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JANUARY, 1923

TECHNICAL PAPER No. 1

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# THE REMOVAL OF SODIUM CARBONATE FROM SOILS

BY

WALTER P. KELLEY AND EDWARD E. THOMAS

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THE REMOVAL OF SODIUM CARBONATE  
FROM SOILS\*

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It is well known that alkali soils which contain sodium carbonate are especially difficult to reclaim. The removal of the excess of soluble salts by leaching is greatly hindered by the deflocculated condition of such soils. The deflocculation increases as the concentration of electrolytes decreases. Consequently after partial leaching the soil may become practically impervious to the penetration of water. The deflocculated condition, however, is by no means the only difficulty to be overcome in the reclamation of the so-called "black-alkali" soils. Perhaps still more important is the fact, as shown in a previous paper,<sup>7</sup> that injurious concentrations of OH-ions may remain in the soil after practically all of the neutral salts have been removed. As a matter of fact, the deflocculated condition of such soils, and also that of certain leached saline soils, previously noted by Scofield and Headley<sup>16</sup> and by Sharp,<sup>18</sup> is probably due primarily to the chemical alkalinity of the soil solution.

Cameron and Patten<sup>2</sup> showed that with certain soils it is possible to leach out practically all of the soluble salts, including sodium carbonate; but as will be discussed more fully in a separate paper by Cummins and Kelley,<sup>4</sup> certain other constituents may be present which are not sufficiently soluble to be removed by ordinary leaching, but which impart injurious alkalinity to the soil solution. The result is that the soil may still be toxic to plants after the principal part of the soluble salts has been removed.

\* Paper No. 90, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.



That black-alkali soils may be improved by special treatment has long been recognized. Early in his investigations on alkali soils, Hilgard recommended the application of gypsum as a means of converting sodium carbonate into calcium carbonate and sodium sulfate. Many years later, Loughridge<sup>14</sup> pointed out that it is necessary to apply approximately twice as much gypsum as is indicated by the analysis of a water extract of the soil. The conclusion of Loughridge is in harmony with recent investigations of this laboratory<sup>7</sup> which show that the total alkalinity is not measured accurately by the prevailing methods of analysis.

Cameron and Seidell<sup>3</sup> and Breazeale<sup>1</sup> concluded that it is not possible to precipitate the carbonate completely, by means of gypsum, where the soil also contains a high concentration of neutral salts. They showed that when equilibrium has been established following the application of gypsum, the concentration of  $\text{CO}_3$  may still remain relatively high.\*

Lipman and Gericke<sup>11</sup> employed manure, Hibbard<sup>6</sup> used manure and other organic materials, and Lipman and Sharp<sup>10</sup> applied sulfuric acid in the treatment of certain black-alkali soils of California. As an outgrowth of his work on the process of sulfonation, J. G. Lipman<sup>12</sup> suggested that the biological oxidation of elemental sulfur might prove to be an efficient means of decomposing sodium carbonate in soils. Recently Hibbard<sup>5</sup> published data which indicate that the application of elemental sulfur may prove to be a practical treatment for black-alkali soils.

In our investigation on the alkali problem of California special attention has been given to the black-alkali soils. Extended laboratory studies are being made on different phases of this subject. A carefully conducted field experiment on a tile-drained area near Fresno, California, is also being made. The results already obtained indicate that it is impracticable to reclaim certain portions of this area by the ordinary leaching process. We have been able to remove the greater part of the neutral salts by flooding the land, but toxic concentrations of sodium carbonate still remain in the soil. Certain plots have also been treated with gypsum followed by heavy flooding. An additional area has recently been treated with elemental sulfur. In connection with these experiments a laboratory study has been made on the effects

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\* The equilibrium between carbonates and neutral salt solutions is being studied further in this laboratory.



of various neutralizing substances, the results of which are presented in this paper.

Five soils have been used in these studies. No. 905 is a fine sandy soil from Riverside, California; 909 is a fine sandy loam from Farmersville, California; 2736 is a clay loam from Salt Lake City, Utah; and 2753 and 2754 are fine sandy loams from Fresno, California. Soil 2754 was taken from the area referred to above, before it was treated with sulfur. Each sample was taken from locations where crops had failed.

TABLE 1  
SOLUBLE CONSTITUENTS OF SOILS

	(Parts per million)				
Laboratory number	905	909	2736	2753	2754
CO <sub>3</sub> .....	210	255	810	1200	420
HCO <sub>3</sub> .....	610	945	656	1281	778
Cl.....	2600	124	4468	1796	1840
SO <sub>4</sub> .....	3555	108	1963	482	788
NO <sub>3</sub> .....	289	265	155	750	419
PO <sub>4</sub> .....	4	23	57	138	73
SiO <sub>2</sub> .....	28	66	75	59	55
Ca.....	5	2	5	7	7
Mg.....	86	12	28	97	22
K.....	336	52	60	52	30
Na.....	3688	644	4745	2860	2370
Total salts.....	12000	2150	12380	9425	6415
pH-value.....	9.4	9.6	9.8+	9.8+	9.8+

#### COMPOSITION OF THE SOILS

The analysis of water extracts of these soils (1:5) is recorded in table 1. It will be noted that the soluble constituents varied considerably, both quantitatively and qualitatively. The total salt content of soils 905 and 2736 was high, that of soils 2753 and 2754 intermediate, and that of soil 909 comparatively low. The content of soluble carbonate, which is of special interest in this connection, also varied considerably, ranging from 210 parts per million in soil 905 to 1200 parts per million in soil 2753. A very large proportion of the soluble salts present was composed of sodium compounds.

Total CO<sub>2</sub> was determined by gently heating samples of these soils with 4 per cent HCl and aerating the CO<sub>2</sub> into KOH-bulbs. The CO<sub>2</sub> equivalent of the soluble CO<sub>3</sub> and HCO<sub>3</sub> was subtracted from the total CO<sub>2</sub> and the remainder calculated as CaCO<sub>3</sub>.

## CALCIUM CARBONATE CONTENT OF SOILS

(Per cent)

Laboratory number	905	909	2736	2753	2754
CaCO <sub>3</sub> .....	2.82	0.60	8.55	0.20	0.16

The results show that soils 2736 and 905 each contain rather high percentages of CaCO<sub>3</sub>, while soil 909 contains a considerable amount and soils 2753 and 2754 much smaller amounts.

## SULFOFICATION EXPERIMENTS

Three different quantities of elemental sulfur were added to equal portions of each soil. After mixing thoroughly, distilled water was added in amounts sufficient to bring the moisture content to the optimum. The several portions were placed in fruit jars and kept at laboratory temperature. After standing for certain periods of time, a part of the soil was withdrawn from each jar and the water-soluble CO<sub>3</sub>, HCO<sub>3</sub>, SO<sub>4</sub> and Ca, and the pH-values were determined. The average results of duplicate experiments are submitted in tables 2, 3, 4, 5 and 6.

TABLE 2  
SULFOFICATION DATA, SOIL 905  
(Parts per million)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 2 weeks						
200	180	678	3589	2	9.3	6
400	150	724	3724	4	9.3	14
800	142	648	3904	10	9.3	15
After 4 weeks						
200	127	610	3951	8	9.0	66
400	105	579	4052	20	8.9	41
800	52	648	4717	42	8.8	48
After 6 weeks						
200	135	533	4147	27	9.0	99
400	97	533	4458	40	8.9	75
800	30	541	5299	75	8.6	73
After 9 weeks						
200	112	533	3828	7	9.0	45
400	75	577	4356	42	8.8	67
800	37	457	5249	100	8.6	71
After 15 weeks						
200	112	404	4134	35	9.2	96
400	60	396	4534	60	8.9	82
800	45	373	5788	132	8.6	93



TABLE 3  
SULFOFICATION DATA, SOIL 909  
(Parts per million)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 2 weeks						
400	157	861	234	2	9.1	10
800	165	839	330	2	9.2	9
1600	142	816	471	2	9.2	8
After 4 weeks						
400	165	823	371	2	9.2	22
800	142	724	531	2	9.0	18
1600	90	686	797	2	8.7	14
After 6 weeks						
400	135	777	452	2	9.3	29
800	120	632	791	2	8.9	28
1600	15	587	1381	2	8.4	27
After 9 weeks						
400	60	709	860	2	8.6	63
800	15	518	1752	2	8.5	68
1600	0	351	3265	287	7.8	66
After 15 weeks						
400	30	610	1013	6	8.5	75
800	0	434	1897	64	7.5	74
1600	0	290	3730	497	7.3	75

TABLE 4  
SULFOFICATION DATA, SOIL 2736  
(Parts per million)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 2 weeks						
1600	810	701	1981	2	9.4	0
3200	862	594	2024	2	9.4	1
6400	645	854	2064	2	9.2	1
After 4 weeks						
1600	412	953	2708	2	9.4	16
3200	315	945	2856	2	9.4	9
6400	165	884	3346	2	9.3	7
After 6 weeks						
1600	217	640	3232	2	9.4	26
3200	105	473	3644	2	9.2	18
6400	15	434	4456	2	8.5	13
After 9 weeks						
1600	105	388	4140	2	8.9	45
3200	0	381	4662	41	7.2	28
6400	0	328	5391	195	8.0	18

TABLE 4—(Continued)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 15 weeks						
1600	45	327	4196	27	8.9	47
3200	0	351	4976	219	7.1	31
6400	0	381	5891	486	7.6	20

TABLE 5

## SULFOFICATION DATA, SOIL, 2753

(Parts per million)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 2 weeks						
1600	1020	1936	705	5	9.6	5
3200	1012	1624	853	10	9.6	4
6400	915	1944	994	4	9.6	3
After 4 weeks						
1600	495	1950	1547	2	9.3	22
3200	442	1273	2458	2	9.3	21
6400	135	693	3855	2	9.0	18
After 6 weeks						
1600	307	1342	2597	5	9.5	44
3200	157	655	3990	2	9.2	37
6400	37	595	4621	2	8.6	22
After 9 weeks						
1600	210	778	3447	3	9.3	62
3200	30	662	4715	11	8.5	44
6400	0	572	5178	60	8.0	24
After 15 weeks						
1600	97	519	3955	7	8.9	72
3200	0	427	4934	66	7.5	46
6400	0	274	6596	457	7.1	32

TABLE 6

## SULFOFICATION DATA, SOIL 2754

(Parts per million)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 2 weeks						
400	225	892	958	2	9.6	14
800	172	778	1388	2	9.3	25
1600	90	747	1759	2	9.1	20
After 4 weeks						
400	172	671	1547	2	9.1	63
800	60	556	2105	2	8.7	55
1600	15	465	2724	2	8.4	40



TABLE 6—(Continued)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 6 weeks						
400	75	701	1734	2	8.8	79
800	0	533	2438	2	7.6	69
1600	0	388	3190	70	7.4	50
After 9 weeks						
400	120	557	1859	2	8.9	89
800	0	510	2675	5	8.2	79
1600	0	343	3439	157	7.2	55
After 15 weeks						
400	142	480	1955	2	8.9	97
800	0	457	2919	56	8.0	89
1600	0	305	4109	355	7.2	69

It will be noted that the sulfur underwent reasonably active oxidation in each soil. Within the first two weeks of the experiment, the soluble CO<sub>3</sub> decreased and the content of SO<sub>4</sub> increased considerably. At the close of the fourth week still further amounts of CO<sub>3</sub> had disappeared and the oxidation process continued throughout the fifteen weeks of the experiment. It is especially interesting that vigorous oxidation of sulfur took place in the presence of comparatively high concentrations of NaCl, Na<sub>2</sub>SO<sub>4</sub> and Na<sub>2</sub>CO<sub>3</sub>.

A relatively high percentage of the added sulfur was finally oxidized in one or more portions of each soil, except 2736. In the latter soil the rate of oxidation was considerably less rapid, possibly because of the relatively high concentration of chloride which it contained.\* With the exception of soil 905, the products resulting from the oxidation of the larger amounts of sulfur finally neutralized the last trace of soluble CO<sub>3</sub> and decomposed a considerable part of the HCO<sub>3</sub> also.

As shown by the pH-values, none of these soils became acid, although the reaction of each soil except 905 finally approached neutrality.

Except in the case of soil 905 almost no calcium was made soluble until practically all of the soluble CO<sub>3</sub> had disappeared. Later, how-

\* It is immaterial, so far as these experiments are concerned, whether biological agencies were solely responsible for the oxidation of the sulfur. (See MacIntire.<sup>15</sup>) The important point is that reasonably active oxidation took place. It is, of course, possible that still more active oxidation might have been induced by inoculating the soil or sulfur with specially active strains of certain species of sulfifying bacteria,<sup>13</sup> but the final effect on the soil would probably not have been materially different.

ever, the solubility of calcium was increased. The fact that small amounts of soluble  $\text{CO}_3$  still remained in soil 905 after substantial amounts of calcium had been dissolved was probably due to the combined effects of the large amounts of  $\text{CaCO}_3$  and neutral sodium salts, especially the sulfate, which this soil contained.

TABLE 7  
EFFECT OF  $\text{H}_2\text{SO}_4$  ON ALKALI SOILS  
(Parts per million)

$\text{H}_2\text{SO}_4$ added	$\text{CO}_3$	$\text{HCO}_3$	Ca	pH
Soil 905				
None	210	610	5	9.4
612	120	1037	100	9.0
1225	60	1403	250	8.6
1633	Trace	1769	302	8.3
2450	0	2348	562	8.2
4900	0	3355	1315	8.0
Soil 909				
None	255	945	2	9.6
612	30	1128	25	9.0
1225	Trace	1357	97	8.3
2450	0	1601	422	7.6
4900	0	1647	1175	7.2
Soil 2736				
None	810	656	5	9.8+
1225	240	1647	70	9.6
1633	6	2120	137	8.3
2450	0	2440	527	8.2
4900	0	2410	1340	7.4
Soil 2753				
None	1200	1281	7	9.8+
1225	210	2379	10	9.6
2205	Trace	2836	32	8.3
2450	0	2882	60	7.9
4900	0	1540	305	7.0
Soil 2754				
None	420	778	7	9.8+
612	105	1067	5	9.4
1102	Trace	1311	20	8.3
1225	0	1434	50	8.1
2450	0	1327	325	7.4
4900	0	152	805	5.8

## EXPERIMENTS WITH SULFURIC ACID

Portions of the same soils were treated with sulfuric acid solutions of various strengths. Two hundred grams of soil were shaken for one hour with 1000 cc. of the solutions. The solutions were then filtered and  $\text{CO}_3$ ,  $\text{HCO}_3$ , Ca and the pH-values were determined in the filtrates.

It will be seen (table 7) that the more dilute solutions of sulfuric acid decomposed only a part of the soluble carbonate, but that the stronger solutions removed the last trace of it. The bicarbonate determinations are especially interesting. They show that with each soil the normal carbonate was first converted into bicarbonate. As the strength of the acid was increased, the bicarbonate was also partially decomposed in soils 2753 and 2754. The fact that the greatest strength of acid used with soils 905, 909 and 2736 yielded extracts with the highest content of  $\text{HCO}_3$ , although  $\text{CO}_3$  disappeared when much weaker solutions were used, was probably due to the  $\text{CaCO}_3$  of these soils. This view derives support from the fact that with soils 2753 and 2754 the  $\text{HCO}_3$  did not increase materially after the soluble  $\text{CO}_3$  disappeared. The pH-values also indicate that  $\text{CaCO}_3$  was involved in these reactions.

The calcium determinations confirm the preceding statements. It will be noted that substantial amounts of calcium were dissolved in soils 905 and 2736 even before all of the soluble  $\text{CO}_3$  disappeared; when only a mere trace of  $\text{CO}_3$  remained in solution still greater amounts of calcium were dissolved in these soils and an appreciable amount in soil 909. Finally, large amounts of calcium were dissolved in each soil by the strongest solutions of sulfuric acid, but the amounts were much greater in soils 905, 909 and 2736 than in soils 2753 and 2754.

## EXPERIMENTS WITH CALCIUM SULFATE

The same soils were also treated with various amounts of calcium sulfate. After adding distilled water in the ratio of 1:5 and shaking for one hour, the extracts were analyzed. The results are shown in table 8.

It was found that as the amount of calcium sulfate was increased, a gradual reduction in the soluble  $\text{CO}_3$  and  $\text{HCO}_3$  took place in each



soil, but that the soluble  $\text{CO}_3$  was completely precipitated in only one soil (909) and in that case only when a large excess of calcium sulfate was added.

These results confirm the conclusion of Breazeale,<sup>1</sup> that it is not possible to precipitate the soluble  $\text{CO}_3$  completely by means of gypsum,

TABLE 8  
EFFECT OF GYPSUM ON ALKALI SOILS  
(Parts per million)

$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ added	$\text{CO}_3$	$\text{HCO}_3$	$\text{SO}_4$	Ca	pH
Soil 905					
None	210	610	3555	5	9.4
2150	135	351	5076	180	9.6
4300	105	259	6110	435	9.6
8600	60	290	8293	1105	9.4
17200	60	213	12256	2430	9.4
Soil 909					
None	255	945	108	2	9.6
2150	120	351	1334	20	9.5
4300	15	274	2402	170	8.4
8600	6	183	4544	822	8.3
17200	0	259	8817	2440	8.0
Soil 2736					
None	810	656	1963	5	9.8+
2150	690	351	3083	67	9.6
4300	555	320	4083	210	9.6
8600	195	183	6365	777	9.6
17200	120	213	10670	2410	9.6
34400	120	183	11948	2880	9.6
Soil 2753					
None	1200	1281	482	7	9.8+
2150	1185	961	1627	35	9.6
4300	1005	1006	2711	70	9.6
8600	510	945	4915	287	9.6
17200	135	839	9204	1800	9.6
34400	120	778	11705	2755	9.5
68800	120	671	11816	2800	9.4
Soil 2754					
None	420	778	788	7	9.8+
2150	330	335	1965	17	9.6
4300	240	351	3053	195	9.6
8600	165	381	5253	985	9.6
17200	135	335	9542	2620	9.4
34400	135	320	10218	2900	9.4

if the soil contains a high concentration of neutral sodium salts. In fact, sufficient amounts of sodium sulfate may be formed as a direct result of the treatment with gypsum, to prevent the complete precipitation of the carbonate.

Since the effect of elemental sulfur is dependent on its first being oxidized to sulfuric acid, and since the neutralizing effects of sulfuric acid and calcium sulfate are both directly proportional to their respective  $\text{SO}_4$  content, it is interesting to compare the effects of these materials on the basis of the  $\text{SO}_4$  actually added to or formed within the soil. Certain parts of the preceding determinations admit of fairly direct comparison on this basis. These show that whereas the smaller amounts of  $\text{H}_2\text{SO}_4$ , whether formed in the soil by the oxidation of sulfur or added as pure solutions, were approximately equally effective in removing normal carbonate, considerably greater amounts of the sulfur oxidation product were required to remove the last trace of it. On the other hand, when chemically equivalent quantities are considered, calcium sulfate proved to be decidedly inferior, both to elemental sulfur and to  $\text{H}_2\text{SO}_4$  solutions, at all stages of the process.

From the preceding discussion it is evident that the effect of oxidizing sulfur was somewhat different from that of sulfuric-acid solutions. With the former, the soil being kept at a moisture content approximating that of good tilth, there was a tendency for both sodium carbonate and sodium bicarbonate to be decomposed simultaneously.\* Except in the case of soil 905 the oxidation of sulfur did not materially increase the solubility of calcium until practically all of the sodium carbonate was decomposed. The sulfuric-acid solutions, on the other hand, caused a marked increase in  $\text{HCO}_3$ , and with certain of these soils calcium was dissolved before all of the soluble carbonate had disappeared.

In the sulfonation series a large part of the  $\text{CO}_2$  set free by the sulfur oxidation products probably escaped into the atmosphere, since the soil moisture must have become saturated with  $\text{CO}_2$  early in the experiment. With the use of sulfuric-acid solutions, on the other hand, normal carbonate was first converted into bicarbonate, just as takes place in the ordinary titration of carbonate solutions.

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\* Since this paper was written, Rudolfs published the results of an investigation on the effect of sulfur on two black-alkali soils. He found that with one soil normal carbonate was first converted into bicarbonate, whereas with the other soil the results were similar to our data. (*Soil Science*, vol. 13, no. 3, pp. 215-229.)

Since certain of these soils contained considerable amounts of  $\text{CaCO}_3$ , the decomposition of the bicarbonate did not set in until a considerable part of the  $\text{CaCO}_3$  had been converted into the bicarbonate also.

#### EFFECT OF CALCIUM SULFATE ON LEACHED PORTIONS OF ALKALI SOILS

A considerable quantity of the same soils was placed on Buchner funnels and leached with distilled water until practically free from chloride and sulfate. The soil was then spread out to dry, and after becoming dry, portions were treated with calcium sulfate as in the preceding experiments. The results are shown in table 9.

Comparing the untreated portions of these soils with the results shown in table 1, it will be seen that in addition to the  $\text{Cl}$  and  $\text{SO}_4$ , a considerable part of the  $\text{CO}_3$  and  $\text{HCO}_3$  was also removed by leaching. Treatment with calcium sulfate after leaching lowered the  $\text{CO}_3$  and  $\text{HCO}_3$  still further, but in contrast to the effects on the unleached soil

TABLE 9  
EFFECT OF CALCIUM SULFATE ON ALKALI SOILS AFTER LEACHING  
(Parts per million)

$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ added	$\text{CO}_3$	$\text{HCO}_3$	$\text{SO}_4$	Ca	pH
Soil 905					
None	75	335	140	30	9.4
2150	30	290	1252	230	8.8
4300	15	259	2420	575	8.4
8600	15	213	4859	1390	8.4
17200	Trace	229	8816	2945	8.3
Soil 2736					
None	240	320	113	5	9.6
2150	45	167	1281	115	8.8
4300	15	167	2501	560	8.4
8600	Trace	152	4907	1525	8.3
17200	0	152	8320	2940	7.4
Soil 2753					
None	420	305	49	10	9.6
2150	60	259	1258	25	9.1
4300	15	213	2451	355	8.5
8600	12	198	4886	1310	8.5
17200	Trace	198	8531	2820	8.3
Soil 2754					
None	45	412	134	5	8.6
537	30	198	335	10	8.9
1075	12	167	649	105	8.3
2150	0	167	1219	305	7.2



(table 8) the application of the largest amounts of calcium sulfate precipitated the normal carbonate completely. It is noteworthy, however, that to produce this effect, relatively large amounts of calcium sulfate were required.

It should be pointed out that the results of these experiments are not in complete harmony with those obtained by the application of gypsum in plot experiments at Fresno, California. The analyses of several hundred soil samples, taken about two months after gypsum had been applied and after the plots had been heavily flooded, have in all cases failed to show any considerable amount of soluble calcium occurring simultaneously with soluble  $\text{CO}_3$ . With the use of calcium sulfate in the laboratory experiments, on the other hand, substantial amounts of both soluble calcium and  $\text{CO}_3$  were found in certain portions of each soil. In the laboratory experiments the products of the reaction remained in the soil, whereas in the plot experiments they were largely leached out. It is probable that the simultaneous occurrence of soluble calcium and  $\text{CO}_3$  noted in the laboratory experiments, was due to the solvent effect of the sodium sulfate formed by the direct action of the calcium sulfate applied.

#### EXPERIMENTS WITH FERROUS SULFATE

As a result of preliminary experiments, Lipman and Sharp<sup>10</sup> suggested that ferrous sulfate might prove to be useful in the treatment of black-alkali soils. Various amounts of this material have been added to unleached portions of these soils. The iron salt was mixed with the soil in the dry state, and then distilled water was added in the ratio of 1:5. After shaking for one hour the solutions were filtered and analyzed, with the results shown in table 10.

It will be noted that ferrous sulfate effectively reduced the alkalinity of each soil. Being a salt of a weak base and strong acid, ferrous sulfate gives an acid solution when dissolved in water. It is interesting to note that the effects produced by this material appear to be similar in nature to those produced by dilute solutions of  $\text{H}_2\text{SO}_4$  (compare tables 7 and 10). The concentration of soluble  $\text{CO}_3$  was reduced by the treatment, while that of  $\text{HCO}_3$  was substantially increased. With those soils relatively high in  $\text{CaCO}_3$  (905 and 2736) the addition of the largest amounts of ferrous sulfate produced the greatest increases in  $\text{HCO}_3$ , but such was not the case with the other soils.

TABLE 10  
EFFECT OF FERROUS SULFATE ON ALKALI SOILS  
(Parts per million)

Ferrous sulfate added	CO <sub>2</sub>	HCO <sub>3</sub>	Ca	pH
Soil 905				
None	210	610	5	9.4
1235	60	701	152	8.6
1875	15	945	200	8.6
3475	0	1113	330	8.1
6950	0	1494	690	8.0
Soil 909				
None	255	945	2	9.6
1735	45	1037	22	9.0
2605	Trace	1128	40	8.2
3475	0	1159	85	8.2
Soil 2736				
None	810	656	5	9.8+
1735	330	1067	12	9.5
3475	15	1540	20	8.6
6950	0	1860	385	8.0
Soil 2753				
None	1200	1281	7	9.8+
3475	285	2181	5	9.6
5210	0	2577	27	7.9
6950	0	2623	45	8.0
13900	0	976	295	7.0
Soil 2754				
None	420	778	7	9.8+
1735	105	1006	5	9.4
2605	15	1128	10	8.3
3475	0	1250	15	8.1
6950	0	1037	317	7.5

Since ferrous sulfate is a by-product in the manufacture of galvanized iron wire and similar materials and one for which present uses are limited, it is possible that this substance might prove to be useful in the practical treatment of black-alkali soils. It is important to state in this connection that an excess of soluble ferrous iron is considered to be extremely toxic to plants. It seems, however, from the limited study given this phase of the subject, that black-alkali soils have the power of precipitating very large amounts of iron, and it is probable that any excess which might be added could be leached into the subsoil and would tend to overcome the alkalinity of the subsoil. Since, as pointed out more fully below, flooding and drainage



is almost as essential to the reclamation of black-alkali soils as is the application of a neutralizing substance, the danger of using an excess of ferrous sulfate may not be great.

When a black-alkali soil is treated with ferrous sulfate a gelatinous precipitate is formed, which probably consists of a mixture of ferrous carbonate, ferrous hydrate and ferrous oxide. While each of these compounds is relatively insoluble in water, it is possible that their solubility in the soil solution might be considerable, especially if large amounts of  $\text{CO}_2$  be present. Under these conditions the concentration of ferrous ions might become sufficiently high to be toxic to plants. In order to gain some light on this point an excess of ferrous sulfate was added to portions of soils 2753 and 2754. After leaching out the excess of salts, it was found that barley seeds germinated readily in each soil, developed an extensive root system and continued to grow normally throughout the brief experimental period (two weeks); in fact, fully as good growth was secured as in portions of the same soils previously leached with a solution of calcium sulfate. Untreated portions of the soils, on the other hand, proved to be extremely toxic to barley, germination having failed in every trial. Moreover, it seems probable that under the conditions prevailing in natural alkali soils, any ferrous iron compounds would soon undergo oxidation with the formation of the less toxic ferric compounds. The iron would probably soon be precipitated as ferric hydrate and ferric oxide. It is planned to test this material more fully in plot experiments.

#### EXPERIMENTS WITH SOLUBLE ALUMINUM

It is well known that trivalent salts, such as those of aluminum, are powerful flocculents for soil colloids. Scofield<sup>17</sup> has suggested the use of aluminum salts as a treatment for certain badly deflocculated soils of the semi-arid region. Since soluble aluminum salts are precipitated as  $\text{Al}(\text{OH})_3$  by alkaline solutions, the alkalinity of the solution being itself reduced at the same time, it is possible that treatment with aluminum salts might prove effective on black-alkali soils. Ordinary potassium alum was used in these experiments. The dry salt was mixed with the soil and the mixture then extracted with water as in the previous experiments. The results are shown in table 11.

TABLE 11  
EFFECT OF ALUM ON ALKALI SOILS  
(Parts per million)

Alum added	CO <sub>3</sub>	HCO <sub>3</sub>	Ca	pH
Soil 905				
None	210	610	5	9.4
742	60	778	140	8.6
900	45	839	145	8.5
2966	0	1235	325	8.2
5932	0	1708	632	8.2
Soil 909				
None	255	945	2	9.6
742	105	1022	15	8.7
1483	15	1159	25	8.4
2966	0	1190	72	8.2
Soil 2736				
None	810	656	5	9.8+
1983	210	1464	30	9.5
2503	105	1616	25	9.0
5932	0	1815	297	8.2
Soil 2753				
None	1200	1281	7	9.8+
2966	315	2486	15	9.5
5932	0	2760	30	8.3
11864	0	1937	40	8.0
Soil 2754				
None	420	778	7	9.8+
742	225	914	10	9.4
1483	75	1159	10	8.8
2966	0	1281	20	8.3

It will be noted that the addition of alum reduced the alkalinity effectively. Since aqueous solutions of aluminum salts are acidic like those of iron salts, their chemical effect on black-alkali soils appears to be of a nature similar to that produced by dilute solutions of H<sub>2</sub>SO<sub>4</sub> and of ferrous sulfate. If the price is not prohibitive these experiments suggest that soluble aluminum salts may possibly find practical application as a treatment for black-alkali soils.

#### EXPERIMENTS WITH CO<sub>2</sub>

Lipman and Sharp<sup>9</sup> showed that the nitrogen-fixing organisms may be quite active in soils that contain comparatively high concentrations



of various sodium salts. Lipman and Gericke<sup>11</sup> found that the application of manure may stimulate the growth of crops on an unproductive black-alkali soil. It has frequently been observed that certain crops, alfalfa in particular, are capable of making reasonably good growth on soils which contain considerable amounts of sodium carbonate, provided the concentration is not too high during the early stages of growth. Since it is well known that the formation of  $\text{CO}_2$  by micro-organisms may be stimulated by the application of manure, and that  $\text{CO}_2$  is given off by the roots of growing plants, it is of interest to study the effects of  $\text{CO}_2$  on black-alkali soils.

Two-hundred-gram portions of the unleached soils were shaken for one hour with 1000 cc. of distilled water that had been partially saturated with  $\text{CO}_2$ . The amounts of  $\text{CO}_2$  contained in the water are recorded as parts per million of the dry soil (table 12).

TABLE 12  
EFFECT OF  $\text{CO}_2$  ON ALKALI SOILS  
(Parts per million)

$\text{CO}_2$ added	$\text{CO}_3$	$\text{HCO}_3$	Ca	pH
Soil 905				
None	210	610	5	9.4
2200	105	1921	125	9.6
4400	45	2486	200	9.0
8800	0	3690	405	8.2
Soil 909				
None	255	945	2	9.6
2200	105	1815	30	9.4
4400	0	2638	60	8.2
Soil 2736				
None	810	656	5	9.8+
2200	420	1998	37	9.6
4400	120	2669	75	9.4
8800	0	5154	130	8.1
Soil 2753				
None	1200	1281	7	9.8+
4400	570	3279	5	9.6
8800	0	5871	80	8.1
Soil 2754				
None	420	778	7	9.8+
2200	135	1693	10	9.5
4400	Trace	2623	20	8.4
8800	0	3538	65	8.0

It will be seen that as the amount of  $\text{CO}_2$  was increased the soluble  $\text{CO}_3$  gradually decreased and finally disappeared from each soil and at the same time the  $\text{HCO}_3$  markedly increased. The OH-ion concentration was reduced by  $\text{CO}_2$ , the extracts of each soil finally giving a pH-value of approximately 8.0. The solubility of calcium was also increased, the increases in the different soils being roughly proportional to their content of  $\text{CaCO}_3$ . It is especially noteworthy that considerable amounts of calcium were dissolved in soils 905 and 2736 without the pH-value of the solution falling below 9.0.

These results suggest, in harmony with Hibbard's data,<sup>6</sup> that the beneficial effect of manure on black-alkali soil may be due, in part at least, to the  $\text{CO}_2$  that is formed in its decomposition, and that the  $\text{CO}_2$  given off by the roots of growing plants may be of some importance in lowering the OH-ion concentration, particularly in that portion of the soil solution which is in contact with the roots.\*

Since a solution of  $\text{NaHCO}_3$  readily passes into  $\text{Na}_2\text{CO}_3$  upon evaporation, and since the transition is hastened by high temperatures such as frequently occur in alkali regions, the effect of manure is likely to be temporary. If the soil contains considerable amounts of  $\text{CaCO}_3$  and if it is leached after the manure has undergone decomposition, the bicarbonate may possibly be washed out with a resulting permanent benefit to the soil.

#### GENERAL DISCUSSION

In the sulfonation experiments the decrease in soluble  $\text{CO}_3$  and  $\text{HCO}_3$  may be ascribed to the action of  $\text{H}_2\text{SO}_4$  formed by the oxidation of the sulfur. Calculating the  $\text{SO}_4$  equivalent of the losses in  $\text{CO}_3$  and  $\text{HCO}_3$  and comparing this quantity with the amount of  $\text{SO}_4$  actually formed, we find that in practically all cases the latter was much greater than the former. With the exception of the early period of the oxidation process, from 75 to 100 per cent more  $\text{SO}_4$  was formed than is required to effect the losses in  $\text{CO}_3$  and  $\text{HCO}_3$  noted. Similarly, the amounts of Ca precipitated by the soil where calcium sulfate was added, were usually at least twice as much as

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\* Pure water at equilibrium with  $\text{CaCO}_3$  with the latter present in excess, gives a reaction as alkaline as pH 8.6 to 8.8, but by merely passing  $\text{CO}_2$  through the suspension the pH-value of the solution can be easily reduced to a point well on the acid side of neutrality. It does not follow, therefore, that the soil solution around the roots of plants growing in calcareous soil in either humid or arid climates is necessarily alkaline.

can be accounted for by the decreases in soluble  $\text{CO}_3$  and  $\text{HCO}_3$ . Soil 905, in fact, appears to have the power of neutralizing  $\text{H}_2\text{SO}_4$  and precipitating Ca in amounts several times as great as would be expected by its soluble  $\text{CO}_3$  and  $\text{HCO}_3$ .

It is commonly held that  $\text{Na}_2\text{CO}_3$  forms adsorption compounds with soils. If such compounds occur in these soils, a part of their ability to neutralize acid and precipitate calcium could be accounted for on this basis. The data obtained by the use of  $\text{H}_2\text{SO}_4$  solutions indicate, however, that a considerable part of the acid was used up by substances other than carbonates at all stages of the reaction.

In a previous paper from this laboratory<sup>8</sup> it was suggested that complex silicates relatively high in sodium may occur in black-alkali soils. Such compounds, if present, would probably react readily with dilute acids. When treated with calcium sulfate the calcium of the added salt would tend to replace sodium, the former passing out of solution and the latter passing into solution. The preceding data strongly suggest that reactions involving double decomposition actually took place in each of these soils, that a part of the added acid was neutralized and a part of the calcium of the calcium sulfate was fixed by substances tentatively designated as alkaline silicates, and that these reactions took place even in the presence of soluble  $\text{CO}_3$ .

Whatever may have been the true nature of the reactions, computations show that the increases in  $\text{HCO}_3$  brought about by the various  $\text{H}_2\text{SO}_4$  solutions were sufficient to account for only a part of the acid used up. This seems difficult to explain if adsorbed carbonate was the only constituent other than soluble carbonate and  $\text{CaCO}_3$  involved in the reaction.

As a result of the several studies that have now been made on the various inter-relationships of salts and soils, it seems clear that black-alkali soils must be regarded as being sodium-saturated soils. Not only do they contain an excess of soluble sodium salts, but the finer fractions of the solid components are predominantly sodium compounds as well. These latter are extremely reactive with various chemical reagents and produce highly alkaline solutions when treated with relatively pure water. A more extended discussion of this phase of alkali soils will be given in a separate paper by Cummins and Kelley.<sup>4</sup>

Since the reactions which take place when black-alkali soils are treated with an acid or with calcium sulfate certainly involve both



the normal carbonate and the bicarbonate of sodium, and probably alkaline silicates and adsorbed carbonates as well, it is safe to conclude that much greater amounts of the neutralizing substance will ordinarily be required than a mere determination of the water-soluble  $\text{CO}_3$  would indicate.

These experiments show that the oxidation products of elemental sulfur and sulfuric-acid solutions lowered the concentration of sodium carbonate similarly in each of the five different alkali soils, but that after a part of the carbonate had been removed the sulfuric-acid solutions were the more effective of the two. With each soil except 905, the sulfur oxidation products finally removed the last trace of soluble  $\text{CO}_3$ . Since, however, these products attacked both normal carbonate and bicarbonate somewhat simultaneously, whereas sulfuric-acid solutions differentiated between these compounds, attacking the latter only after the former had been decomposed, a considerably greater amount of the sulfur oxidation products than of the sulfuric acid was required to remove the last trace of soluble  $\text{CO}_3$ .

While calcium sulfate lowered the concentration of soluble  $\text{CO}_3$  in each soil, the experimental results indicate that it is not possible to precipitate the carbonate completely with this material, so long as the concentration of neutral sodium salts remains excessive. Similar conclusions have recently been drawn by Hibbard.<sup>6</sup> Moreover, the results suggest that soils high in black alkali, although practically free from neutral salts, may also require leaching. Unless the soil be leached, the concentration of sodium sulfate, formed as a result of the treatment, may become so high as to hold a considerable part of the  $\text{CO}_3$  in solution.

As bearing on the practical application of these results, it is important to remember that crops produce a much deeper root system when grown in semi-arid than in humid regions. Not infrequently the roots of annuals penetrate to a depth of three or more feet and the roots of perennials, alfalfa and fruit trees for example, commonly grow to a depth of several feet. Moreover, the ability to develop a deep root system seems to be essential to the well-being of the plant. If the subsoil relatively near the surface is impermeable or uncongenial to root development, the growth of crops is likely to be seriously restricted. As is well known, black-alkali soils are usually underlaid by excessively alkaline subsoils, and a high degree of alkalinity may occur in successive layers of the subsoil to a depth of several feet. In

addition, one or more of the neutral salts are commonly present in black-alkali soils in toxic concentrations.

From the above discussion it seems evident that the reclamation of black-alkali soils is likely to involve at least two steps: (1) the application of a neutralizing substance in quantity sufficient to overcome the alkalinity of the soil and of the subsoil to the depth necessary for the normal development of the crops to be grown; (2) flooding and drainage. If elemental sulfur or sulfuric acid be applied to the surface of a soil in amounts sufficient to neutralize the injurious alkalinity present in both the soil and the subsoil, an acid condition of the surface soil must inevitably result unless other alkaline substances such as  $\text{CaCO}_3$  are present. On the other hand, an excess of gypsum may be applied to the surface and leached downward, thus ameliorating the subsoil without affecting the surface soil adversely.

Finally, as already pointed out, the silicates that naturally occur in black-alkali soils are probably saturated with sodium, the readily replaceable calcium having already been substituted by sodium in nature. Treating such a soil with an acid, though it may effectively neutralize the alkali carbonates, cannot restore the calcium to the soil silicates, and there is much evidence that certain calcium silicates play an extremely important rôle in normal soil processes. There can be but little doubt that soil silicates saturated with calcium, or largely so, possess superior physical properties and promote conditions especially favorable for normal plant growth.

In view of these facts, we believe that gypsum, although considerably less effective chemically as a means of removing soluble carbonate, may in the long run prove to be preferable to elemental sulfur or sulfuric acid as a treatment for non-calcareous black-alkali soils. The fact that enormous deposits of gypsum occur in numerous places not far distant from the areas of black-alkali soils is also a factor to be considered. With alkali soils which contain an excess of  $\text{CaCO}_3$ , or in which the injurious alkalinity does not extend into the subsoil, it is possible that elemental sulfur or sulfuric acid may prove quite as effective as gypsum, and in some cases even more so. It is also possible that a treatment consisting of a combination of elemental sulfur and gypsum may prove superior to either alone.

It was also found that both ferrous sulfate and alum effectively neutralized the alkalinity of each soil, but the determination of the practical value of these materials necessitates further investigation.

## SUMMARY

1. Elemental sulfur undergoes reasonably active oxidation in alkali soils which contain relatively high concentrations of various sodium salts. It was found that the sulfur oxidation products finally neutralized the last trace of soluble  $\text{CO}_2$  in every soil studied except one.

2. With the use of sulfuric-acid solutions, normal carbonate was first converted into bicarbonate, whereas the sulfur oxidation products decomposed normal carbonate and bicarbonate simultaneously.

3. The addition of calcium sulfate produced a substantial lowering of the soluble  $\text{CO}_2$ , but it failed to reduce the alkalinity completely except when used in conjunction with leaching.

4. When considered on the basis of chemical equivalents, sulfuric-acid solutions were somewhat more effective than the sulfur oxidation products and considerably more so than calcium sulfate. The results indicate that each of these materials reacts in black-alkali soils with substances other than carbonates. The sulfur oxidation products and calcium sulfate also decompose bicarbonates, and each appears to react with alkaline silicates as well.

5. The amount of these materials that must be added is apparently considerably greater than a determination of the water-soluble alkalinity would indicate, and the excess appears to vary in different soils.

6. Since injurious alkalinity commonly occurs in the subsoil as well as in the surface soil, the successful treatment of black-alkali soils involves the necessity of considering the subsoil. Gypsum, although less effective chemically than elemental sulfur or sulfuric acid, may nevertheless be preferable in practice, since it is frequently necessary to employ leaching and the alkaline silicates present are capable of being converted into calcium silicates by the action of calcium salts. These latter silicates probably perform highly important functions in soils.

7. Both ferrous sulfate and alum neutralized the  $\text{CO}_2$  in each soil. The results indicate that either of these materials might be useful in the treatment of black-alkali soil. An excess of soluble ferrous iron or aluminum is, however, considered to be toxic to plants. Moreover, the precipitate formed by each of these materials



is gelatinous and may produce undesirable physical properties, especially where large amounts of black alkali occur. Culture experiments extending over a period of two weeks indicate, however, that the soil after treatment with ferrous sulfate and leaching, may be a favorable medium for growth. Further studies must be made before the practical value of these materials can be said to be established.

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TECHNICAL PAPER No. 2

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THE CITRUS NEMATODE, *TYLENCHULUS*  
*SEMIPENETRANS*

BY

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## INTRODUCTION

Citrus trees were formerly considered to be free from the attack of nematodes. J. C. Neal<sup>5</sup> claimed to have found the root-knot nematode, *Heterodera radicicola*, on citrus roots in Florida, but this observation has not as yet been confirmed. Gaston Lavergne<sup>4</sup> reported a nematode *Anguilula*, much larger but similar to *Heterodera radicicola*, on lemon trees in Chili, which were very badly injured by an excessive amount of water. Small knots were found on the roots in which the young nematodes developed.

In the early part of 1912 the writer's attention was called to a parasitic nematode found on the roots of citrus trees by J. R. Hodges, Horticultural Inspector of Los Angeles County, California. In the fall of the same year a study of this subject was undertaken and a preliminary report was issued by the writer<sup>6</sup> on the prevalence and distribution of the nematode in the citrus districts of California. Soon after this report was published, N. A. Cobb<sup>2</sup> of the United States Department of Agriculture, determined the life history of the citrus nematode and gave it the name *Tylenchulus semipenetrans*. Within six months this nematode was found infesting citrus roots grown in Alabama and Florida, and Cobb<sup>3</sup> reported having found it on citrus roots obtained from Florida, Spain, Malta, Palestine, Australia and South America. Trabut<sup>7</sup> reported having found the same nematode on citrus roots in Algeria.

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\* Paper No. 96, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

Cobb reported almost one hundred species of nematodes in the soil around the roots of citrus trees, many of which have larval forms closely resembling those of the *Tylenchulus semipenetrans*, and which might be confused with it. The characteristics of this species, however, are clearly defined and once they are known there is little difficulty in establishing its identity.

#### APPEARANCE AND HABITS OF *TYLENCHULUS SEMIPENETRANS*

Cobb<sup>2</sup> describes this species as follows: "Cuticle naked, traversed by 400-500 plain transverse striae. Neck cylindroid, becoming convex-conoid near the continuous head, which is rounded in front. No lips, amphids, or eye-spots. Spear and oesophagus typically tylenchoid. Median bulb elipsoidal, with valve; posterior swelling pyriform to elongated, without valve. Male tail conoid to the somewhat blunt terminus. Posterior part of the adult female saccate, with wide blunt tail bent toward the ventral side. Vulva in the midst of a prominent ventral suture."

Both male and female nematodes bear a spear with its acute point in the mouth. This spear is hollow and connected with a narrow tube which passes through the oesophagus to a sucking bulb or pump by which great suction may be exerted upon the surface of the tissues. The nematode obtains its nourishment by placing the mouth parts on the surface of the rootlet, thrusting the spear into the tissue and drawing out the fluid content of the cell by means of the sucking bulb.

The oral spear of the male *Tylenchulus semipenetrans* is not so highly developed as that of the female. In the male it also seems to deteriorate with age, which indicates that the males are less capable of attacking a healthy rootlet than are the females. Some of the former have been found attached to citrus roots, but it is possible that they may have entered the tissues which were already broken down by the females.

Because of this method of feeding, the anterior portion of the body of the female is thrust into the growing tissues of the root, the head having been found piercing to a depth of eight or ten cells. The posterior egg-bearing portion of the female remains outside of the root and the eggs, which are relatively large and thin-shelled, are deposited on the outside of the root and are encased in a gelatinous material. The larvae, when they emerge from the egg, are colorless



and often almost transparent. At an early age the females insert their heads into citrus rootlets as above described, and probably remain in this position throughout their life cycle. A retreat from the root would be almost impossible, since in many instances the body becomes enlarged somewhat after the outside layers of the root have been penetrated.

The stationary existence of the females accounts for the large number of nematodes found on a small area of root. One hundred female nematodes were found imbedded in the tissue of a piece of citrus root 4 mm. long, while 108 were found on another root section of the same length.

The life cycle of the nematode may be completed in from six to eight weeks, and as each female is capable of depositing a large number of eggs, their rate of increase is very rapid.

The organism attacks the small feeding rootlets of citrus trees near the surface of the ground and down as far as the rootlets occur, having been found at a depth of twelve feet. *Tylenchulus semipenetrans*, in contrast to the root-knot or garden nematode, *Heterodera radicumicola*, does not form knots or enlargements of any kind on the root and does not entirely enter the root. The root-knot nematode attacks a large number of plants and the beet nematode, *Heterodera schachtii*, is often found on plants other than beets, while *Tylenchulus semipenetrans*, as far as known, attacks only citrus plants. The roots of a great many plants growing in infested citrus groves have been carefully examined, but in no instance were the citrus nematodes found attacking any rootlets other than citrus.

#### APPEARANCE OF INFESTED TREES

The above-ground portion of the citrus tree may or may not show ill effects from the attacks of the nematode. Trees only slightly infested were found, which to all appearances were in perfect condition. However, among the hundreds of trees examined, every *heavily infested* tree showed evidence of injury. Badly infested trees have the appearance of being undernourished, the leaves are small and slightly yellow or mottled, the fruits small and often unmarketable, the whole tree being stunted and showing a marked state of deterioration. This condition has been found many times with trees which previously had been healthy and vigorous and which formerly produced good crops of fruit.

While the above-ground portion of the trees may show the effects of the nematode, the greatest injury is found on the small feeding rootlets. Although this is not always a reliable indication, the soil particles commonly cling to infested roots, from which they are not readily removed. This is due to the gelatinous material in which the eggs are imbedded. In many cases the writer has been unable to remove the soil completely by washing in water, even with the aid of a moderately fine hairbrush. Very frequently, when a rootlet with closely adhering soil particles is examined under the microscope, a colony of female *Tylenchulus semipenetrans* will be found attached to it.

A noticeable symptom of the nematode attack, which is always present where large numbers occur, is the separation of the bark of the rootlet from the inner woody portion. Whenever badly infested roots are dug up and the soil is shaken from them, the outer portion of the tissue is often found to have been removed, leaving the white woody cylinder. Close examination reveals the fact that the bark is easily removed from a large percentage of the infested rootlets, which is not true of healthy citrus roots. Healthy growing tips are seldom found on badly infested roots and very often various fungi which are always present in the soil, find access to the broken-down tissues.

### SURVEY

Groves have been examined for *Tylenchulus semipenetrans* in all of the principal citrus districts, from Butte and Glenn counties in the north to San Diego County in the south. As a rule, the organism is most prevalent in the older citrus sections. In one district comprising several thousand acres, over 75 per cent of the groves were examined without finding one grove that was free from the nematode. In other districts, large portions of the groves appear to be free from the pest, or at most are only slightly infested. In practically every badly infested district, the poor trees were found to be infested with the nematode. From this survey it is fairly certain that the attack of the citrus nematode presents a problem of considerable economic importance.

It is a well-known fact that the root-knot nematode thrives best in loose, sandy soil. Bessey<sup>1</sup> states that plants badly infested with the root-knot nematode are often able to free themselves of the

organism when transplanted into a heavy soil. As a result of investigations on different types of soil, it was found that the citrus nematode occurs more abundantly in loose sandy soil, but no soil type was found to be free from it.

#### MEANS OF DISTRIBUTION

*Tylenchulus semipenetrans* is very easily and quickly scattered by various methods. One of the most important of these is through the transportation of nursery stock. When small trees from an infested nursery are transplanted to an orchard, they harbor the nematodes and thus infest soil which otherwise may be free from the organism. In order to prove this definitely, the following investigation was made.

In 1912 a large company in Glenn County planted lemon trees from five nurseries and orange trees from two nurseries. In 1913 they planted lemon trees from sixteen nurseries, orange trees from thirteen nurseries, and a few grapefruit trees from two nurseries. All of the nurseries from which the trees were secured were from 300 to 500 miles distant from Glenn County.

Careful examination of the trees secured from the different nurseries was made. The results showed that the trees from infested nurseries remained infested when transplanted, while those from other nurseries in which no infestation occurred were free from the nematode. Furthermore, it has been found that *Tylenchulus semipenetrans* may be carried to an orchard even though the trees are planted with bare roots.

Water may also be a means of distributing the nematode. Irrigation water running under an infested tree may carry nematode-infested soil to other trees, and storm water may carry the nematode from one grove to another. In sections where the run of the storm water was carefully followed, the nematodes were found to be carried from the grove where the initial infestation occurred to every grove which lay in the path of the storm water for a distance of two miles. Canals into which the waste water from irrigated groves may run, and from which water is used for further irrigation, may also be a source of infestation.

While nursery trees and running water are probably the principal means of spreading the citrus nematode, it may be carried from place to place by farm implements, such as tractors, plows, cultivators and



wagons, by horses, or even by persons. In fact, anything which carries moist soil from one place to another, may be a means of distributing the nematode.

#### EFFECT OF *TYLENCHULUS SEMIPENETRANS* ON CITRUS TREES

The following experiments were conducted to determine whether injurious results follow the attack of *Tylenchulus semipenetrans*.

*Experiment I.*—Two water-tight boxes,  $8 \times 10 \times 1\frac{1}{2}$  feet, were constructed, each being provided with two drain pipes,  $3\frac{1}{2}$  feet long. The boxes were sunk in the ground to a depth of 16 inches, thus allowing two inches to remain above the surrounding soil. By this arrangement, the soil in the boxes was protected from outside contamination. Soil of a sandy type was obtained from a section where the nematode had not been found. After carefully mixing, the soil was sterilized with carbon bisulfid applied in six holes per square yard of surface. The holes were made 8 to 12 inches deep and 8 ounces of carbon bisulfid was applied to each square yard. As soon as the carbon bisulfid was poured into the holes, they were closed with soil. The boxes were then left for eighteen days, in order to permit diffusion of the gas throughout the soil and its later dissipation into the air.

Water was then added to the soil and 50 sweet-orange seedlings, *Citrus sinensis* Osbeck, and 50 sour-orange seedlings, *C. aurantium* L., were planted in each box. These seedlings had been examined very carefully and were found to be free from nematodes and in a healthy, normal condition. The plants in the two boxes were given identical treatment until they began to make a healthy, vigorous growth. Then box A was irrigated with tap water, while box B was irrigated with water which had previously passed over citrus roots that were heavily infested with the nematode. As a result, box A was kept free from the nematode, while box B became infested.

The trees which were kept free from the nematode made much better growth than the trees which were infested. Twenty trees of the infested lot died within two years, but only one of the uninfested.

*Experiment II.*—Instead of using boxes containing a large number of trees, as in Experiment I, 16-inch pots were used in which individual trees were planted. The soil used was of the same type as in

the previous experiment, but instead of sterilization with carbon bisulfid, steam sterilization was used. Thrifty, nematode-free nursery trees of  $\frac{5}{8}$  inch diameter, budded on sour-orange stock from six to eight inches above the ground, were planted in the pots. These consisted of 40 Navel orange trees, *Citrus sinensis* Osbeck; 20 Valencia, *C. sinensis* Osbeck; 20 grapefruit, *C. grandis* Osbeck; and 20 Eureka lemon trees, *C. limonia* Osbeck. In addition, the following varieties of citrus seed were planted in 6-inch pots: sour orange, bitter orange, both *C. aurantium* L.; sweet orange, *C. sinensis* Osbeck; grapefruit, *C. grandis* Osbeck; Ponderosa, rough lemon, Eureka lemon, Lisbon lemon, all *C. limonia* Osbeck; lime, *C. aurantifolia* Swingle; trifoliate orange, *Poncirus trifoliata* Raf.; kumquat, Marumi, *Fortunella japonica* Swingle; and kumquat, Nagami, *Fortunella margarita* Swingle.

Every precaution was taken to guard the trees from outside infection, and the conditions were as nearly uniform as possible. A cage was constructed of 6-foot wire netting of 1-inch mesh and six tables, each  $3 \times 30$  feet, were placed in the enclosure. The tops of the tables were 12 inches from the ground and a  $1 \times 8$  redwood board was placed around them. The pots were then placed on the tables and the spaces between them filled with sawdust and shavings in order to reduce the amount of evaporation from the pots. By this arrangement the danger from infection from surrounding soil or water was eliminated.

The trees were given uniform treatment until they had begun to make healthy vigorous growth. Then the soil in one-half of the pots was infested with nematodes by irrigating with water previously brought in contact with infested roots. The control trees were watered with tap water. In every other respect the pots were given similar treatment.

Within five weeks after the first inoculation, nematodes were found attacking the growing roots of several of the trees. The treatment with infested water was continued until every tree in this part of the experiment was found to be attacked by the organism.

For a time the infested trees were as vigorous and apparently as healthy as the uninfested trees, but gradually the former began to show signs of deterioration. The trees were allowed to grow for four years, when the root systems of all but twenty were examined. After

the tables had been removed from the enclosure, twenty of the large trees and fifty of the small trees from each lot were transplanted to plots.

The difference in the appearance of the infested and non-infested trees is shown in plates 2 to 8, inclusive. The tops of the infested trees were stunted and the leaves were scanty, small, and many of them yellow. All of the roots were in poor condition and many of them were dead. The uninfested trees showed unmistakable thrift, the root systems entirely filling the pots. The trees which were transplanted continued to show a difference in growth and vigor after having been taken from the pots.

In these experiments 1240 trees, one-half of them infested with the nematode, and one-half not infested, were grown under controlled conditions. Without exception, the trees not infested with the nematode made better and more normal growth than the infested trees. This clearly demonstrates that citrus trees may be definitely injured by the citrus nematode, *Tylenchulus semipenetrans*.

*Experiment III.—The relation of the nematode injury to the attack of Fusarium.*

Small fibrous rootlets were obtained from twenty-six groves located in widely distributed citrus districts. Fifteen of these groves were infested with nematodes, while the remaining eleven were not. The rootlets were carefully washed and partially sterilized by treating with  $\frac{1}{10}$  per cent mercuric chloride solution for two minutes, then placed in corn-meal agar. Fungus developed on 49.5 per cent of the roots from the infested groves, and on 19.6 per cent of the roots from the groves free from the nematode attack. All of the fungus which developed proved to be the same species of *Fusarium*.

In order to study the effect of the fungus attack under controlled conditions, wooden boxes 12 inches deep, the sides measuring 12 × 13 inches at the top and 12 × 11 inches at the bottom, were constructed. One side was made of glass, which was placed in a groove one inch from the end of the sloping side and was inserted in such a manner that it could easily be removed in order to permit observation of undisturbed roots. This glass was covered with a removable board which kept out the light and also served as a protection for the glass. The boxes were filled with soil in which were planted sweet-orange, sour-orange, grapefruit and Lisbon lemon seedlings. The seedlings were



allowed to grow undisturbed until the roots had come in contact with the glass. The glass was then removed and the roots were inoculated without pricking them, with a culture of *Fusarium* fungus which had been isolated from orange roots. No injury resulted to the roots. The roots were then pricked and the *Fusarium* was introduced into the tissues of the roots, but the trees continued to show a healthy vigorous growth without signs of injury. This indicates that healthy citrus rootlets are not easily injured by an attack of *Fusarium*.

# EXPERIMENTS IN THE CONTROL OF TYLENCHULUS SEMIPENETRANS

*Effect of applying chemicals to the soil.*—An orange grove was selected in which the trees were six to eight years old, all of them badly infested with the nematode, and in poor condition. Seventeen rows of six trees each were used. The first fifteen rows were Navel oranges, all very uniform, while the last two rows were St. Michael oranges, slightly larger and more thrifty than the Navels. The soil was of a sandy type, typical of the district in which the grove was

TABLE 1

KINDS AND AMOUNTS OF MATERIALS APPLIED TO THE SOIL SURROUNDING  
NEMATODE INFESTED TREES IN A GROVE

Sixteen rows of six trees each were treated.

Row	Material	Pounds to Each Tree <sup>1</sup>					
		Tree No. 1	2	3	4	5	6
1	Lime.....	70	70	40	40	20	20
2	Liver of sulfur .....	20	20	10	10	5	5
3	Flowers of sulfur .....	20	20	10	10	5	5
4	Ammonium sulfate.....	25	25	15	15	10	10
5	Copper sulfate.....	20	20	10	10	5	5
6	Lead arsenate.....	2	2	1¼	1¼	½	½
7	Potassium hydrate.....	20	20	10	10	5	5
8	Formaldehyde.....	1	1	½	½	¼	¼
9	Arsenic.....	2	2	1¼	1¼	½	½
10	Carbon bisulfid.....	45*	45*	30*	30*	15*	15*
11	Calcium carbide.....	12	12	8	8	4	4
12	Potassium cyanide.....	1	1	½	½	¼	¼
13	Mercuric chloride.....	1½	1½	¾	¾	½	½
14	Carbolic acid.....	15	15	10	10	5	5
15	Chloral hydrate.....	34†*	17†*	0	0	15	6
16	Iron sulfate.....	45	45	30	30	15	15

<sup>1</sup> Amounts marked "\*" given in ounces.

† Nicotine sulfate.

located. A basin from fourteen to sixteen feet across was made around each tree. The trees were irrigated by filling this basin with water. Table 1 indicates the chemicals used and the amount applied to each tree. All of the chemicals, with the exception of carbon bisulfid, were applied on August 18, 1914, and were added with the water at the time of irrigation. The mercuric chloride and potassium cyanide were dissolved in warm water before pouring them into the basins. The water in the basin was thoroughly stirred after the chemicals were applied, in order to insure even distribution around the tree.

The carbon bisulfid was applied on September 22, in 144 holes per tree. The holes were 18 inches apart and alternately 10 and 15 inches deep. The holes were made with a King soil tube and each hole was immediately filled with soil as soon as the carbon bisulfid had been poured into it. On October 14, carbon bisulfid was again applied to trees 1, 3 and 5, in row 10. The trees received the same application as in the previous treatment with the difference that 169 holes were made per tree at the rate of nine holes per square yard of surface.

The following chemicals produced no noticeable effect on the trees: lime, liver of sulfur, flowers of sulfur, ammonium sulfate, lead arsenate, potassium hydrate, formaldehyde, arsenic, potassium cyanide, mercuric chloride, carbolic acid, nicotine sulfate and iron sulfate. Definite injury to the trees was produced by copper sulfate, carbon bisulfid, calcium carbide and chloral hydrate, especially where the larger amounts were used.

All of the trees treated with copper sulfate were injured. Two of these were almost killed, while several of the branches died on the others. The injury was roughly proportional to the amount of copper sulfate applied. In many cases certain branches were killed and on four of the trees gum exuded from the trunk. Large strips of bark were killed where the injury was severe. The copper sulfate entered the roots and passed up the trunk into the branches.

The larger amounts of carbon bisulfid almost killed the trees. No gumming was noticed as in the case of the copper sulfate, but the fibrous roots were practically all killed. The fruit on these trees turned yellow very soon after the application of the carbon bisulfid, and the leaves wilted and dropped off.

Very little damage was produced by the calcium carbide. On two of the trees gum exuded from the bark in a few places, but without any further noticeable effects.

Chloral hydrate had a very decided effect, especially upon the tree which received the larger application. Gum exuded from the bark in a great many cases. The leaves uniformly turned brown and died at the tip, and when badly affected they dropped off. The tree receiving the smaller amount of chloral hydrate was similarly affected, but only a few of the leaves were injured sufficiently to cause them to drop.

Though some of these treatments were so severe that the trees were injured and some practically killed, none of them controlled the nematode. In fact, it was evident that in practically every case the number of nematodes was not reduced by the treatment. On the trees where the small fibrous rootlets were injured, the number of nematodes was reduced, but the effect on the organism may have been indirect.

*Effect of fertilizers.*—It has been suggested by Bessey<sup>1</sup> and a number of other investigators, that the bad effects of the root-knot nematode may be overcome by the use of fertilizers. In some places practically complete eradication has been effected by this means.

With this thought in mind, roots obtained from the trees on the fertilizer plots of the Citrus Experiment Station were carefully examined. In this experiment there are twenty-two plots, some of which have been fertilized with mineral fertilizers applied singly or in combination, one with manure, one with manure and rock phosphate. Two of the plots have been covercropped in addition to an application of manure and rock phosphate, while three of the plots have not been fertilized.

At the time of making the examination the trees were eight years old and the fertilizers had been applied for seven consecutive seasons.

Two samples of roots were taken, one from the north side and one from the south side of each of eight trees in each plot. In all cases nematodes were found. Some of the same trees were inspected from time to time until the trees reached the age of thirteen years, and always with the same results. This would indicate that treatments of this nature are ineffective in controlling the nematode on citrus trees.



TABLE 2

THE CHEMICALS USED IN THE TREATMENT OF NURSERY TREES, THE CONDITION OF THE TREE AFTER TREATMENT, AND THE EFFECT UPON THE NEMATODES

Concentration of chemical	Time	Condition of tree	Nematodes found
MERCURIC CHLORIDE			
.1%.....	5 min.	Dead	Large number
.1%.....	10 min.	Dead	Large number
.5%.....	5 min.	Injured	Large number
.5%.....	10 min.	Dead	Large number
NICOTINE SULFATE			
1 to 1000.....	5 min.	Good growth	Large number
1 to 1000.....	10 min.	Good growth	Large number
1 to 500.....	5 min.	Good growth	Large number
1 to 500.....	10 min.	Good growth	Large number
1 to 100.....	5 min.	Good growth	Large number
1 to 100.....	10 min.	Good growth	Large number
CARBON BISULFID			
1 oz. per 15 cu. ft.....	5 min.	Good growth	Large number
1 oz. per 15 cu. ft.....	10 min.	Good growth	Large number
2 oz. per 15 cu. ft.....	20 min.	Dead	Large number
2 oz. per 25 cu. ft.....	30 min.	Good growth	Large number
4 oz. per 25 cu. ft.....	30 min.	Good growth	Large number
8 oz. per 25 cu. ft.....	30 min.	Good growth	Large number
FORMALIN			
.1%.....	5 min.	Good growth	Large number
.1%.....	10 min.	Good growth	Large number
.1%.....	20 min.	Good growth	Large number
.5%.....	5 min.	Dead	Large number
.5%.....	10 min.	Injured	Large number
.5%.....	20 min.	Dead	
1%.....	5 min.	Dead	
1%.....	10 min.	Dead	
1%.....	20 min.	Dead	
POTASSIUM CYANIDE			
.1%.....	1 min.	Good growth	Large number
.1%.....	5 min.	Good growth	Large number
.1%.....	10 min.	Good growth	Large number
.5%.....	10 min.	Good growth	Large number
1%.....	1 min.	Good growth	Large number
1%.....	5 min.	Good growth	Large number
1%.....	10 min.	Good growth	Large number
2%.....	1 min.	Good growth	Large number
2%.....	5 min.	Injured	Large number
2%.....	10 min.	Good growth	Large number

TABLE 2—(Continued)

Concentration of chemical	Time	Condition of tree	Nematodes found
ACETIC ACID			
.5%.....	10 min.	Good growth	Large number
1.5%.....	10 min.	Good growth	Large number
3%.....	10 min.	Injured	Large number
CITRIC ACID			
.5%.....	10 min.	Good growth	Large number
1.5%.....	10 min.	Good growth	Large number
3%.....	10 min.	Good growth	Large number
FUMIGATION WITH HYDROCYANIC ACID			
1/10 schedule.....	1 hr.	Dead	Large number
1/8 schedule.....	1 hr.	Dead	Large number
1/8 schedule.....	30 min.	Dead	Large number
1/4 schedule.....	30 min.	Top killed	Large number
1/2 schedule.....	30 min.	Dead	
Full schedule.....	30 min.	Dead	
CHECK TREES, NO TREATMENT			
		Good growth	Large number

CONTROL OF *TYLENCHULUS SEMIPENETRANS* ON NURSERY TREES

In the experiments on the control of the nematode on nursery stock, the roots of the trees were carefully washed with water in order to remove all of the soil possible. They were dipped in a solution of various chemicals for definite lengths of time, then washed with water in order to remove the chemical and thus check its action on the root tissues. After this treatment the trees were planted in soil which was free from the nematode. The chemical used and its effect on the tree, together with the effect on the nematode, are given in table 2.

Some of the trees were also fumigated with hydrocyanic acid before being transplanted. In this case, the whole tree was placed in a fumigation box and was fumigated as shown in table 2. The trees were then planted in the usual way. The fact that the roots became dry during the fumigation process may have caused the death of so many of the trees with this treatment. In the carbon bisulfid treatment the roots were exposed to the gas in a tight can for the time specified.

By referring to the table it will be noted that all of the trees which made good growth were infested with the nematode, also that a number which were badly injured and later died were found to be infested. The roots of these latter trees were examined for nematodes a few days after treatment and again at the time when the leaves began to wilt. Live nematodes were found on and around the roots. However, after the trees had died and there were no live rootlets on which the nematodes could feed, living nematodes were not found. This shows that the treatments which were severe enough to kill the trees did not destroy all the nematodes.

The best results were obtained by dipping the roots in water at 130° F. for 20 seconds, and for 30 seconds. Nematodes were found on the roots of the trees treated in this manner, but they were not so numerous as on the roots of the trees treated with water at a lower temperature (table 3). Trees from one nursery treated with water at 135° F. for 20 seconds were killed, while those obtained from another nursery were not injured. The trees obtained from the first nursery were in a less vigorous condition than those obtained from the second. However, all of the trees used as checks in each lot, grew, sending out new shoots soon after being transplanted. This indicates that the hot-water treatment was near the danger line, and the vigor of the tree may be the deciding factor as to the effect upon the tree.

From the results obtained in these experiments, it was thought that good results might be obtained by cutting off most, if not all, of the fibrous roots of the young trees and carefully washing the remainder in order to remove all of the soil clinging to the roots. In this experiment varying degrees of pruning were tried. Some trees were severely pruned, by removing all of the small roots; others moderately pruned, by cutting off a considerable percentage of the fibrous roots; others slightly pruned; and still others not pruned at all. The roots of these trees were then dipped in water at the temperature and for the length of time which gave the best results in the former experiments.

With certain trees the hot-water treatment was repeated after the lapse of three or four weeks. In this way the nematode eggs not killed in the first treatment might be given time to hatch and then be killed by the second treatment. On May 18 and 19, 1916, some of the trees in each set were treated with hot water, packed in damp



TABLE 3

TIME OF TREATMENT OF THE ROOTS OF TREES WITH HOT WATER, THE PERCENTAGE OF THE TREES WHICH GREW, AND THE EFFECT UPON THE NEMATODES

Ten trees in each treatment.

Temperature of water	Time	Percentage of trees growing	Nematodes found
110° F.....	30 sec.	100	Large number
	1 min.	100	Large number
	2 min.	100	Large number
	5 min.	100	Large number
	10 min.	100	Large number
115° F.....	30 sec.	100	Large number
	1 min.	100	Large number
	2 min.	100	Large number
120° F.....	20 sec.	100	Large number
	30 sec.	100	Large number
	1 min.	100	Large number
	5 min.	100	Large number
	10 min.	100 but very poor growth	Large number
125° F.....	1 sec.	100	Large number
	5 sec.	100	Large number
	10 sec.	100	Large number
	20 sec.	100	Large number
	30 sec.	100	Large number
	1 min.	100	Large number
130° F.....	1 sec.	100	Large number
	5 sec.	100	Large number
	10 sec.	100	Large number
	20 sec.	100	Small number
	30 sec.	100	Small number
	1 min.	0	
135° F.....	1 sec.	100	Large number
	5 sec.	100	Large number
	10 sec.	90	Large number
	20 sec.	80	Small number
140° F.....	1 sec.	100	Small number
	5 sec.	20	Small number
	10 sec.	0	
	20 sec.	0	
150° F.....	1 sec.	0	
Check trees, no treatment.....		100	Large number

sphagnum, and stored in a cool place for 25 days. On June 12 and 13, 1916, they were again given the same treatment and then planted in the soil. A table of the results follows (table 4):

TABLE 4

TIME OF TREATMENT OF THE ROOTS OF TREES WITH HOT WATER, THE PERCENTAGE OF THE TREES WHICH GREW, AND THE EFFECT UPON THE NEMATODES UNDER DIFFERENT METHODS OF ROOT PRUNING

Ten trees in each treatment, 25 check trees.

Temperature	Time	Percentages of trees growing			
		Roots pruned severely	Roots pruned moderately	Roots pruned slightly	Roots not pruned
ONE TREATMENT					
130° F.....	20 sec.	30*	40*	60*	80*
	30 sec.	30*	20	50*	0
	45 sec.	20*	20	30	0
135° F.....	10 sec.	0	10*	40*	20*
	20 sec.	0	40	20	20*
TWO TREATMENTS					
130° F.....	20 sec.	20	40	40*	20
	30 sec.	0	20	40*	0
	45 sec.	0	40	0	10
135° F.....	10 sec.	40	40	40	100
	20 sec.	0	0	0	60
	30 sec.	0	0	20	60
	45 sec.	0	0	0	20
Check trees, no treatment.....		0	30*	65*	100*

\* Female nematodes found alive.

We note that all of the trees which were severely pruned and planted without treatment with the hot water, died; while 30 per cent of those moderately pruned, 65 per cent of those slightly pruned, and 100 per cent of the unpruned trees lived. With trees in good condition, this percentage might have been different, but it is a fact that trees badly infested with the nematode are usually in poor condition and they cannot withstand the injury caused by pruning off or killing most, if not all, of the fibrous roots.

A study of the tabulated results indicates that none of these treatments can be used as a means of controlling the nematode; the temperature necessary to kill all of the eggs and larva of the nematode is

too near the danger point for the tree. However, it is clearly shown that the number of the nematodes was greatly reduced by these treatments.

#### INFESTED SOIL.

In the control of the nematode it is very desirable to know how long the organism can live in the soil after the infested plants have been removed. Two ten-acre tracts of land were studied, each bearing two-year-old peach and walnut trees and on which severely infested citrus trees had been grown previously. Owing to their situation, the water from the surrounding nematode-infested citrus groves was excluded.

Young citrus trees free from nematodes were planted in the soil near several of the peach and walnut trees on March 12, 1915. On March 23, 1916, the roots were examined and nematodes were found on 50 per cent of the trees. On January 4, 1917, they were again examined and all of the trees were then found to be infested.

These observations justify the conclusion that two years is not sufficient time for the soil to become free from the citrus nematode. A contributing factor is found in the fact that many of the citrus roots remain alive in the soil for a considerable time after the tree has been taken out. These roots, if infested, will harbor the nematode as long as they live. The results of this experiment, therefore, do not make it possible to conclude definitely as to the length of time the citrus nematode can survive in the soil.

#### SUMMARY

This investigation shows that the citrus nematode, *Tylenchulus semipenetrans*, is an injurious parasite. It is similar to the beet nematode (*Heterodera schachtii*), especially in its manner of attack. In each case the organism pierces small rootlets while the posterior portion of the body remains outside of the root. In this respect it differs from the root-knot nematode (*Heterodera radiculicola*).

The root-knot nematode attacks a great many plants and the beet nematode is known to attack plants other than beets, while up to the present time the citrus nematode has not been found on any roots other than those of citrus.



The citrus nematode attacks the small fibrous rootlets of citrus trees and when the tree is badly infested a very large percentage of the small rootlets are involved. The injury to the fibrous roots of the tree necessarily lowers the vitality of the entire tree and the above-ground portion appears to be undernourished.

By growing nursery trees in pots under controlled conditions it has been definitely proven that the root development and the growth of the top may be greatly retarded by the nematode. In a number of instances the trees were killed, while in other cases a portion of the top was killed and the remainder stunted.

The entire fibrous portion of the root system of trees grown in pots became infested with the nematode, and this has also been found to be true of trees growing in badly infested groves. It seems safe to conclude that a bad infestation of the citrus nematode can scarcely fail to be injurious to the citrus tree. Among the hundreds of trees examined, no *badly infested* tree was found to be in good condition.

Citrus nematodes are very easily distributed by various methods. They may be carried from grove to grove in earth clinging to implements, the hoofs of animals, etc. In other instances they are conveyed in soil which is carried from place to place by storm water. They are also distributed in the soil adhering to the roots of infested nursery stock. The latter method is the most important as nursery trees are very often infested, and they carry the nematode to the groves in which they are planted. Great care should be taken to keep the nursery stock free from an infestation of the nematode. The nursery trees should be grown on uninfested soil, in order that new citrus plantings may be free from an attack of the parasite.

When we consider the possible control of this parasite we find that any treatment used for its eradication must necessarily reach the whole root system on account of the fact that all of the roots may be infested. Various chemicals have been used, but no practical method of eradication has as yet been found. Of the different methods that have been tried, the best result was obtained with the use of hot water, but the temperature necessary to kill all of the nematodes and eggs lies very close to the danger point for citrus trees.

When the organism once becomes established on the roots of citrus trees, there does not appear to be any satisfactory means of removing it.\*

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\* The writer wishes to acknowledge his indebtedness to Dr. H. S. Fawcett and Dr. W. P. Kelley, of the Citrus Experiment Station, for many valuable suggestions.

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PLATE 1

Fig. 1. *Tylenchulus semipenetrans*. Cross-section of a citrus rootlet showing a female with her head permanently embedded in the root tissues.

Fig. 2. *Tylenchulus semipenetrans*. Female in cross-section of citrus rootlet. Root tissue injured by nematode attack.



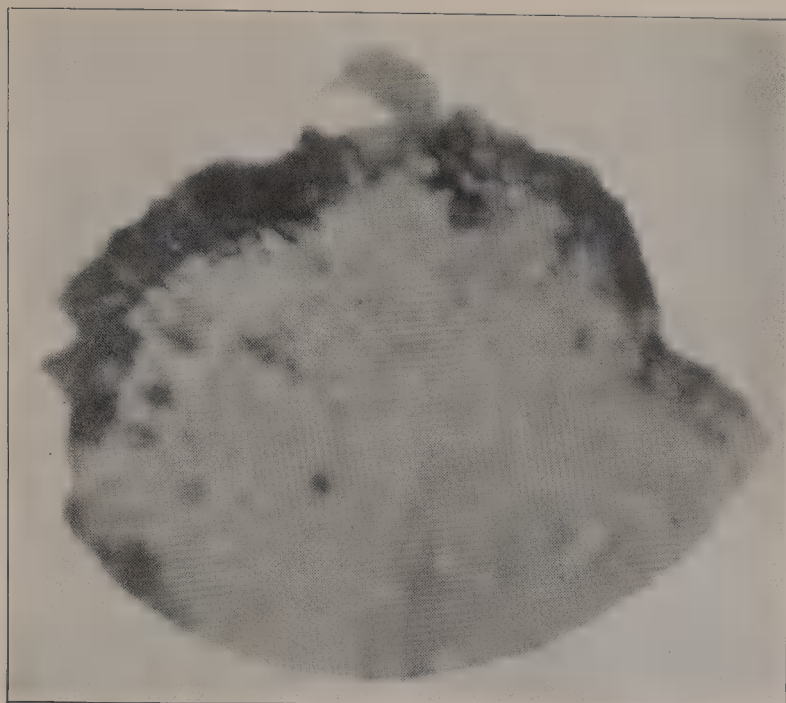


Fig. 1

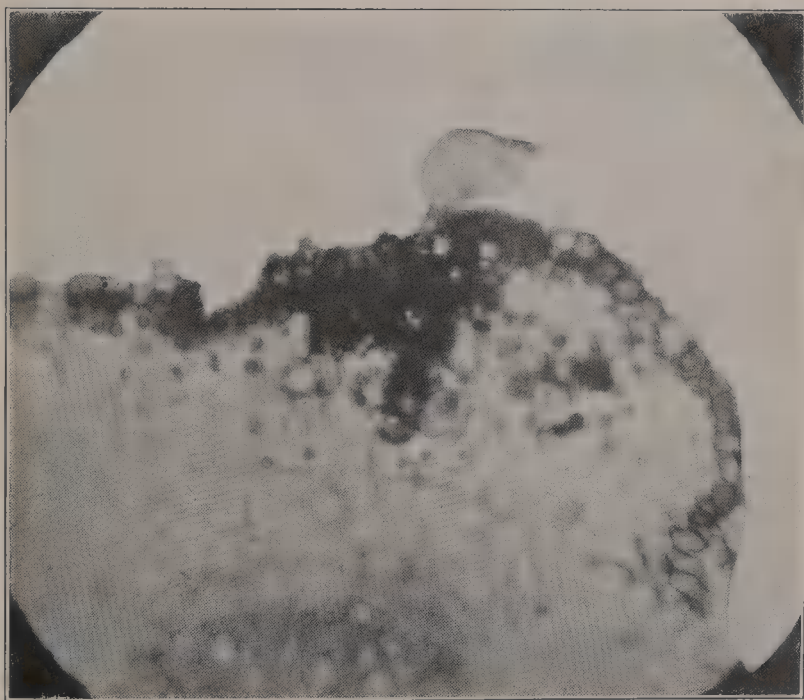


Fig. 2







PLATE 2

Fig. 1. Grapefruit trees. The tree on the left was infested with *Tylenchulus semipenetrans*, the one on the right was not.



Fig. 1







PLATE 3

Fig. 1. Naval orange trees. The one on the left was infested with *Tylenchulus semipenetrans*, the one on the right was not. The trees were grown in 16-inch pots.



Fig. 1







PLATE 4

Fig. 1. Eureka lemon trees grown in 16-inch pots. The roots were infested with *Tylenchulus semipenetrans*.



Fig. 1







PLATE 5

Fig. 1. Two Eureka lemon trees grown in 16-inch pots. These trees were not infested with *Tylenchulus semipenetrans*. Compare with Plate IV, figure 1.



Fig. 1







PLATE 6

Fig. 1. Sour-orange seedlings. The four large seedlings were not infested, the four small seedlings were infested with *Tylenchulus semipenetrans*.



Fig. 1







PLATE 7

Fig. 1. Large nursery trees in pots. Not infested with *Tylenchulus semi-penetrans*.



Fig. 1







PLATE 8

Fig. 1. Large nursery trees in pots. Infested with *Tylenchulus semipenetrans*. A portion of the branches are dead. Compare with Plate VII, figure 1.



Fig. 1





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THE FORMATION OF SODIUM CARBONATE  
IN SOILS\*

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ARTHUR B. CUMMINS AND WALTER P. KELLEY

These studies were first suggested some years ago by a field and laboratory comparison of the soils from the various plots of a fertilizer experiment at the Citrus Experiment Station, Riverside, California. The plot which has been treated with sodium nitrate annually for fifteen years has for several years possessed markedly different physical properties from those of adjacent plots. The soil, especially near the end of the irrigation runs, has become relatively impermeable, hard when dry and puddled when wet. It was suggested that the change in the soil consisted substantially in the displacement of some of the calcium in the soil silicates by the sodium of the  $\text{NaNO}_3$  applied, and that these new sodium silicate combinations were either colloidal in nature, or that conditions were suitable for their hydrolysis, whereby alkalinity has been produced with its attendant deflocculating effect upon the clay particles. Laboratory studies on the soil from this plot showed that it has indeed undergone marked physical modification. The rate of percolation of water downward, the rise of water by capillarity, etc., were much slower than with the soil from adjacent plots that have not been treated with sodium salts.

In attempting to prepare soil comparable with that of the  $\text{NaNO}_3$  plot, virgin soil taken nearby was first artificially leached in the laboratory with solutions containing neutral sodium salts and then with pure water to remove the excess of electrolyte. Almost immediately after

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\* Paper No. 100, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

the water was applied, the rates of percolation decreased, the soil masses swelled and dark-colored, alkaline percolates were obtained.\* Frequently these percolates became quite turbid with finely divided suspended matter, and after some days percolation usually ceased entirely. The soil residuum after such treatment presented a sterile, "baked" appearance upon drying, not unlike natural alkaline soils *in situ*.

Recently similar observations have been reported from other laboratories. It is interesting to note that very similar ideas have been developed by totally independent workers. The earlier literature contains many references to isolated aspects of the subject, but only a few of these need be reviewed here.

Van Bemmelen<sup>44</sup> noted a decrease in the rate of percolation when salts were washed from clay, hydrated oxides of tin, silica, etc., and that the dispersion of the colloids was very marked. He reported that the reaction was reversible; that is, the colloids could be flocculated again by treatment with salts. Mayer<sup>23</sup> had previously noted that treatment with either NaOH or NaCl and then with water, produced similar puddling effects in natural soil, whereas  $\text{Ca}(\text{OH})_2$  caused flocculation. Warrington<sup>46</sup> called attention to the fact that leaching an acid-treated soil with water results in a similarly dispersed system. Hissink<sup>16</sup> reported that both KCl and NaCl decreased the permeability of the soil. Sharp<sup>40</sup> noted a very marked puddling effect produced by leaching salts from soils in tanks, and concluded that the physical effects produced were largely due to the markedly colloidal properties of the sodium compounds formed in the soil.

In a study previously reported on the chemical effect of salts on soils, data were presented confirming the well known replacement of bases between soils and neutral salt solutions.<sup>19</sup> A stoichiometric relationship was found to hold between the cation removed from a salt solution and the bases set free from the soil. This relationship can be expressed both graphically and mathematically, and when so expressed conforms with the established laws governing replacement and some metathetical reactions.

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\* The chemical nature of the dark-colored extracts which are obtained from natural alkaline soils, and which are produced so abundantly in semi-arid soils by artificial means, has been insufficiently studied. The solutions are colloidal and contain much organic matter, but the relationship of the latter to the chemistry of alkali soils is not clear at present. This, and other aspects of the subject are being studied further at the Citrus Experiment Station.

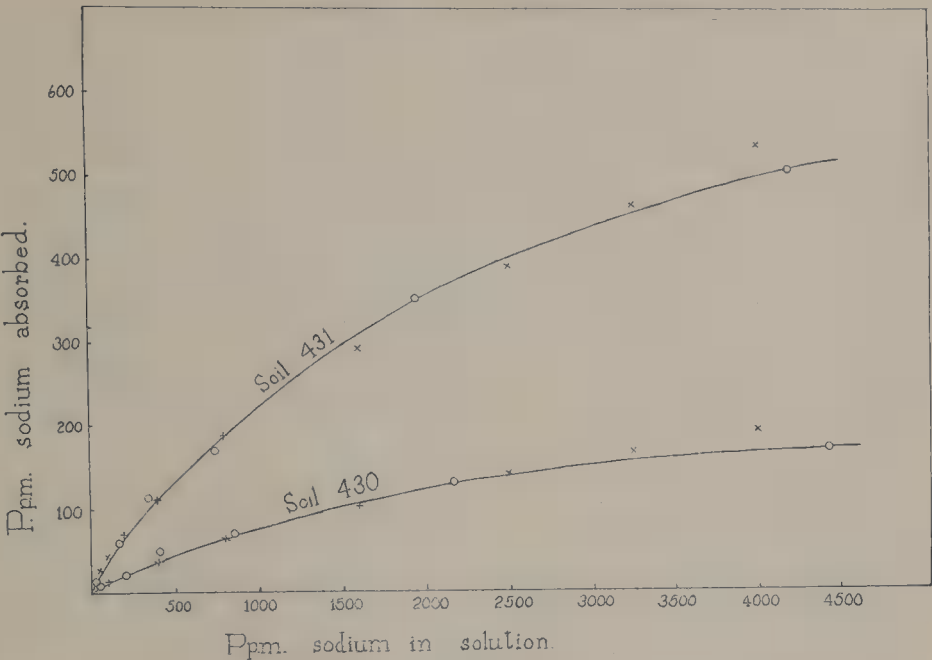


Fig. 1.

Curves showing the relation between sodium absorbed and sodium in solution at equilibrium. The circles represent the actual experimental data; the crosses represent the values calculated from the equations referred to in the text.

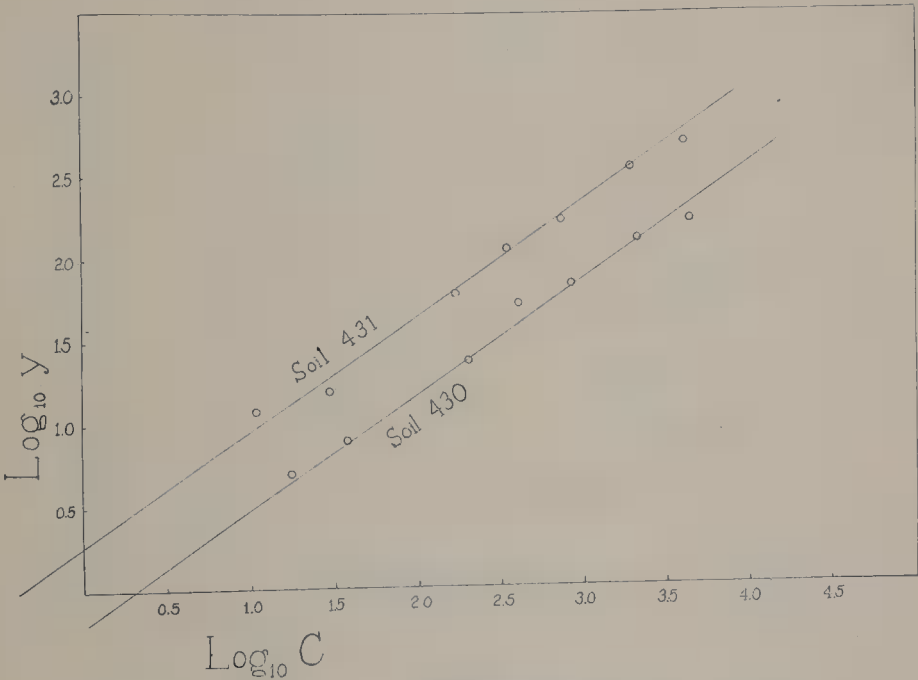


Fig. 2

Curves of figure 1 plotted logarithmically.



For example, when a soil was shaken to equilibrium with a solution of sodium chlorid, the sodium fixed was found to be an exponential function of the concentration of the sodium salt in the solution at equilibrium. As the total amount of calcium and the other bases brought into solution was found to be chemically equivalent to the sodium fixed, it is evident that the bases replaced are likewise a function of the concentration of the sodium salt. This confirms the work of Eichhorn,<sup>11</sup> Van Bemmelen,<sup>45</sup> Lemberg,<sup>21</sup> Wiegner,<sup>47</sup> Rice,<sup>36</sup> Sullivan,<sup>42</sup> and many others who have shown that basic exchange is a very general phenomenon in soils as well as in a wide variety of other substances.

The results obtained with two soils from southern California are shown graphically in figure 1. The exponential nature of the curves and their similarity are shown more clearly by plotting logarithmically, whereby two practically parallel straight lines are obtained.

The empirical equations expressing the relations between the sodium fixed and the concentration of the sodium chlorid in solution at equilibrium, are for the two soils respectively:  $\frac{y}{m} = 1.905 C^{0.68}$  and  $\frac{y}{m} = 0.631 C^{0.69}$ ; where  $y$  = the amount of sodium fixed,  $m$  = the weight of soil, and  $C$  = the concentration of sodium chlorid in solution at equilibrium.\* Freundlich<sup>12</sup> has emphasized the general applicability of this type of equation for a wide variety of adsorption phenomena and has styled the curves of such equations "adsorption isotherms," since they express the relationships of such reactions as the adsorption of iodine by starch, picric acid by silk, etc. Some soil investigators (<sup>15, 24, 34</sup>) have concluded, therefore, that basic exchanges between salt solutions and soils are essentially processes of physical adsorption. A review of numerous and varied chemical reactions, however, shows that many purely chemical reactions proceed in a similar manner.

In 1884 Ostwald<sup>33</sup> showed that the solubility of alkaline earth sulfates in acids is an exponential function of the concentration of the acid. Hall and Gimingham<sup>14</sup> showed the same relationship in the

\* The equations were obtained by plotting the values of the  $\log [NaCl]$  versus the  $\log [Na]$  fixed. From the approximately straight lines thus obtained, the constants  $k$  and  $1/p$  in the general equation  $\frac{y}{m} = kC^{1/p}$  are estimated. The values of  $k$  are given by the intercept of the straight lines on the  $y$ -axis, and the value of the exponential constants  $1/p$  are numerically equal to the slopes of the curves.

replacement of bases by ammonia in soils. Prescott<sup>35</sup> also demonstrated a similar relationship in the reaction of oxalic acid with soils in the presence of dilute nitric acid. The general applicability of the exponential curve to biological phenomena has been shown by Moore and Bigland.<sup>30</sup> Most conclusive of all are Meyerhoffer's<sup>26, 27, 28</sup> extensive investigations on reciprocal salt pairs. He studied simple systems, such as  $K_2CO_3 + BaSO_4$ , and other metathetical equilibria, and found the reactions of many such purely chemical changes to follow exponential curves.

It is concluded, therefore, without discussing the nice differences between physical adsorption and chemical combination, that such typical basic exchanges as take place between a soil and a salt solution are primarily chemical in nature in the sense that the exchange of bases is stoichiometric in character and that the products of the reaction do not show marked differences from ordinary chemical compounds.

If we consider the reaction between a soil and a salt solution to be chemical, then the soil must undergo a change reciprocal in nature to that taking place in the solution. That is, as calcium replaces part of the sodium in the solution, sodium replaces part of the calcium in the soil. Essentially the process consists in the building up of a system of solid particles relatively richer in sodium and poorer in calcium (also Mg and K) than that originally present in the soil. Although the actual masses of the bases replaced are small in comparison with the total soil mass, the changes are sufficiently marked to produce profound physical difference in the soil. This difference is especially evident when the soil mass is freed from the excess of electrolyte. Whereas the calcium-silicate system is only slightly soluble and relatively stable, the sodium-silicate system is unstable, easily hydrolyzable and apparently capable of existing in the colloidal state.

In the paper cited<sup>19</sup> it was also noted that some soils may give distinctly alkaline extracts after treatment with neutral salts.\* The

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\* As is well known, many soils in humid regions impart an acid reaction to neutral salt solutions. Replacement of bases has been shown to be quite as common with these as with soils from arid regions; but, whereas calcium, magnesium, and potassium are the principal bases replaced from both alike, monovalent salts apparently also replace aluminum, iron, and manganese from certain acid soils of humid regions, but not from semi-arid soils. It has been claimed, although not definitely proven, that the acidity of saline extracts of the former is due largely to the aluminum, iron, etc., brought into solution by the neutral salt. The fact that salt solutions effect different soils differently in regard to iron, aluminum, etc., suggests a difference in the fundamental structure and chemical nature of the compounds present.

production of alkalinity was so pronounced when certain experimental details were observed, that further work has been done to determine the origin of this alkalinity. While the exact nature of the chemical reactions involved has not been thoroughly established and further study is being devoted to the pure chemistry of the phenomena, a preliminary discussion of the problem seems desirable. It appears that similar processes occurring in nature may be responsible for much of the carbonate alkalinity in arid lands. Furthermore, the theory as developed later offers a rational explanation for the conversion of some saline soils into alkaline soils.

Recently Scofield and Headley<sup>39</sup> published a paper on the physical condition of soils in relation to the alkali problem, considering especially the impermeability of soils to water produced in the course of reclamation by flooding and draining. These authors present data showing that neutral sodium salts produce three marked effects, namely: (1) the rate of percolation of water through soils is reduced by previous treatment with salts; (2) the percolate becomes turbid; (3) the percolate becomes strongly alkaline. The authors emphasized the deterioration produced in the physical properties of saline soils when the soluble salts are leached out by flooding or irrigation, but they neglected to discuss the possibility that such reactions are a potent agency in the generation of sodium carbonate. They confirm the facts that the alkalinity is developed before all the neutral sodium salts are leached from the soil and that the rate of percolation declines sharply upon leaching with water. They also emphasize the fact that the addition of calcium or aluminum salts is an effective means of ameliorating or preventing the action of sodium salts.

Later Scofield<sup>38</sup> emphasized the occurrence of colloidal sodium silicates as important components of the salt complex of arid soils and suggested the use of soluble aluminum compounds in correcting the physical properties of soils rich in such sodium compounds. He concluded that relatively large amounts of aluminum salts may be added without danger of rendering the soil toxic.

Earlier papers by Mondésir,<sup>29</sup> Gedroits,<sup>13\*</sup> and Dominicus<sup>10</sup> came to our attention after this work was well advanced. These papers are generally unavailable to American workers and deserve more detailed review because of the experimental and theoretical contributions to

the phenomena in question. Bobko<sup>3</sup> has later confirmed some of Gedroits' conclusions.\*

Mondésir investigated certain calcareous soils in France where sodium chlorid was brought to the land by sea winds, and found that 80 per cent of the total chlorids of a water extract was present as calcium chlorid. He concluded that the sodium of the sodium chlorid had been fixed by the soil, calcium having been set free, and that the resulting calcium chlorid was removed by rains. The sodium absorption compounds were then capable of reacting with calcium bicarbonate, the calcium taking the place of the absorbed sodium. In this way sodium bicarbonate was generated. Mondésir produced experimentally 100 grams of trona ( $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ ) from one kilogram of another calcareous soil, by twenty repetitions of the treatment, first with a solution of  $\text{NaCl}$ , then with water, and finally with a solution of  $\text{CO}_2$ . He apparently did not consider the possibility of the formation of sodium carbonate in the absence of calcium carbonate, nor that the sodium absorption compounds may themselves hydrolyze, yielding alkaline solutions.

Gedroits observed that alkalinity is developed in saline soils upon leaching out the salts with relatively pure water, and considered that  $\text{CaCO}_3$  plays an important part in the process. The rôle ascribed to  $\text{CaCO}_3$  is, however, different from the metathetical reaction wherein  $2\text{NaCl} + \text{CaCO}_3 = \text{Na}_2\text{CO}_3 + \text{CaCl}_2$ .† He considers this reaction to be of limited importance as a source of  $\text{Na}_2\text{CO}_3$  in soils. Rather, he concludes, " $\text{NaCl}$ ,  $\text{Na}_2\text{SO}_4$  and  $\text{CaCO}_3$  are not to be considered as primary sources of soda (sodium carbonate) in the soil; but the rôle of these salts is an intermediate one. Sodium replaces other bases (Ca, Mg, K) from the humates and silicates of the soil, saturating the compounds to a greater or less degree with sodium, and it is these new sodium compounds that function as the direct source of  $\text{Na}_2\text{CO}_3$ , since they dissolve and decompose in the soil solution, giving rise to small amounts of alkalinity. Under suitable conditions, large amounts of alkalinity may be produced by reacting with  $\text{CaCO}_3$ ."

\* A paper entitled "Base exchange and alkalinity in Egyptian soils," by J. A. Prescott, published in *Cairo Scientific Jour.*, vol. 10, nos. 106 and 107, pp. 58-64, 1922, was received after our manuscript had been written. This paper contains an interesting discussion of this problem and verifies the general conclusions drawn herein. It also refers to several important investigations by European and African workers which had previously escaped our attention.

† Breazeale<sup>4</sup> has more recently discussed this reaction at considerable length.



On the basis of this theory, Gedroits suggests that alkaline soils arise from saline soils. The neutral salts first saturate the soil to a greater or less degree with sodium, at which point the formation of significant amounts of sodium carbonate and the structure characteristic of alkaline soils are hindered by an excess of these salts. If, however, the concentration of these salts undergoes strong reduction, the saline soil then changes into an alkaline soil, the hydrolytic products combining with  $\text{CaCO}_3$  under suitable conditions to form large amounts of  $\text{Na}_2\text{CO}_3$ .

In the practical reclamation of saline and alkaline soils he concludes that artificial leaching alone can accomplish its purpose with neither class of soil. Saline soils would indeed deteriorate by it, since the tendency would be for their conversion into alkaline soils. Finally, he concludes that a combination of artificial leaching with the addition of gypsum should suffice, but that a considerable excess of gypsum above that recommended by Hilgard is necessary in the case of black-alkali soils; that is, an amount of calcium sufficient to replace the sodium in the absorption compounds as well as to neutralize any sodium carbonate that may be already present.\*

Dominicis, like Gedroits, obtained phenolphthalein alkalinity by washing the excess of salt from sodium-saturated soils and demonstrated that the amount of finely divided suspended matter was closely correlated with the amount of alkalinity. He furthermore stated that these reactions are responsible for the origin of sodium carbonate in nature.

Dominicis' theoretical explanation of the process is as follows: sodium replaces calcium, magnesium, etc., in certain silicate and humate combinations in the soil, forming sodium "absorbati" or "absorbates."† which are colloidal in nature; strong electrolytes (for

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\* Loughridge<sup>22</sup> in 1897, in enumerating some of the reasons why gypsum has failed in the reclamation of some "black-alkali" soils, concluded that twice the theoretical amount of gypsum should be applied. It is doubtful whether the reason for this was known at that time. Apparently Loughridge considered that there must be more sodium carbonate present than can be determined directly. As pointed out by Kelley and Brown,<sup>28</sup> it is not always possible to estimate the amount of sodium carbonate in a soil by ordinary solution methods of analysis. However, the theory of Gedroits and the experiments herein reported suggest the need for a still greater amount of gypsum. [See <sup>20</sup>.]

† The term "absorbate," suggested by Dr. H. S. Reed in translating the Italian word "absorbati," is retained in this paper as a convenient means of referring to the reactive Na-Ca-silicate complexes occurring in arid soils. It is realized that the expression has no definite significance as an acid radical, in the sense of nitrate, sulphate, etc., but is retained until the chemical nature of the compounds can be established.



example, sodium chlorid) keep the sodium "absorbates" coagulated as hydro-gels in which form hydrolysis does not take place. Therefore, no alkalinity can be developed until the concentration of electrolyte is materially reduced. In the absence of electrolytes the "gels" pass into "sols." The sodium then dissociates and forms sodium hydroxide, which is the primary source of the alkalinity. This sodium hydroxide may be converted into sodium carbonate by the action of calcium carbonate, but the presence of limestone is not essential, since the conversion into carbonate readily takes place in its absence. In nature, the equilibrium between dissociated sodium and the non-dissociated "absorbates" is continually displaced because of the transformation of the hydrate into the carbonate by  $\text{CO}_2$ . The passage of the hydro-gels into hydro-sols and the action of the negative charge of the hydroxyl ions in dispersing the colloid and maintaining the stability of the dispersion, explain the deflocculation effects accompanying the formation of alkalinity and the phenomena by which alkaline soils may either become impermeable to water or be reduced almost to sand through the removal of the clay by erosion.

The essential differences in the theories of Gedroits and Dominicis are thus slight. Gedroits apparently considers that the hydro-gels can, in themselves, hydrolyze, and that calcium carbonate is necessary for any appreciable formation of sodium carbonate. Dominicis believes that only the hydro-sols are directly concerned in the formation of alkalinity and that the presence of calcium carbonate is immaterial. It should be pointed out that Dominicis performed his experiments with a soil which originally contained considerable amounts of  $\text{CaCO}_3$  but he is of the opinion, although no definite proof is given, that the  $\text{CaCO}_3$  was completely dissolved and removed from the soil by the preliminary extraction with neutral salts. In the light of the results reported below, it seems possible, however, that  $\text{CaCO}_3$  may have been responsible either directly or indirectly for a considerable part of the alkalinity which Dominicis noted.

#### EXPERIMENTAL RESULTS

##### *The rôle of $\text{CaCO}_3$ in the hydrolysis of sodium "absorbates" in the soil*

One-hundred-gram portions of two different soils, each of which contained no  $\text{CaCO}_3$ , were shaken (with amendments as noted) in

flasks with liter portions of N/5 NaCl solutions. The suspensions were allowed to settle out and the supernatant liquids siphoned off. These treatments were repeated five times, when pure water was substituted for the salt solutions and similar treatments continued. Tables 1 and 2 show the amounts of phenolphthalein alkalinity found in the supernatant liquids.

TABLE 1  
ALKALINITY DEVELOPED BY EXTRACTING SOIL 430 WITH N/5 NaCl,  
THEN WITH H<sub>2</sub>O

Preliminary treatment	Secondary treatment	Order of secondary treatments cc. N/50 acid per liter (phenolphthalein)					
		1st	2nd	3rd	4th	5th	6th
Soil saturated with N/5 NaCl .....	H <sub>2</sub> O	Trace	0	0	0	0	0
Soil + CaCO <sub>3</sub> saturated with N/5 NaCl .....	H <sub>2</sub> O	3.7	19.0	24.5	20.0	10.3	3.1
Soil saturated with N/5 NaCl .....	CaCO <sub>3</sub> + H <sub>2</sub> O	1.0	12.0	16.4	12.0	5.5	3.2
Soil saturated with N/5 NaCl .....	CaCO <sub>3</sub> + N/5 NaCl	0	0	4.5*	26.0	24.5	16.0
Soil leached with H <sub>2</sub> O .....	H <sub>2</sub> O	0	0	0	0	0	0

\* After the second treatment H<sub>2</sub>O was substituted for N/5 NaCl.

TABLE 2  
ALKALINITY DEVELOPED BY EXTRACTING SOIL 431 WITH N/5 NaCl,  
THEN WITH H<sub>2</sub>O

Preliminary treatment	Secondary treatment	Order of secondary treatments cc. N/50 acid per liter (phenolphthalein)						
		1st	2nd	3rd	4th	5th	6th	7th
Soil saturated with N/5 NaCl .....	H <sub>2</sub> O	0	0	0	0	0	0	0
Soil + CaCO <sub>3</sub> saturated with N/5 NaCl .....	H <sub>2</sub> O	0	0	9.0	22.6	27.0	22.2	19.0
Soil saturated with N/5 NaCl .....	CaCO <sub>3</sub> + H <sub>2</sub> O	0	0	9.3	17.3	25.0	20.3	17.4
Soil saturated with N/5 NaCl .....	CaCO <sub>3</sub> + N/5 NaCl	0	0	0*	0	11.7	27.0	28.5
Soil leached with H <sub>2</sub> O .....	H <sub>2</sub> O	0	0	0	0	0	0	0

\* After the second treatment H<sub>2</sub>O was substituted for N/5 NaCl.

Similar studies were made with Na<sub>2</sub>SO<sub>4</sub> and NaNO<sub>3</sub>. The results were similar to those obtained with NaCl, as is shown in tables 3 and 4.

TABLE 3  
ALKALINITY DEVELOPED BY EXTRACTING SOIL 430\* WITH N/5  $\text{Na}_2\text{SO}_4$ ,  
THEN WITH  $\text{H}_2\text{O}$

Preliminary treatment	Secondary treatment	Order of secondary treatments cc. N/50 HCl per liter (phenolphthalein)						
		1st	2nd	3rd	4th	5th	6th	7th
Soil saturated with N/5 $\text{Na}_2\text{SO}_4$ .....	$\text{H}_2\text{O}$	0	0	0	0	0	0	0
Soil + $\text{CaCO}_3$ saturated with N/5 $\text{Na}_2\text{SO}_4$ .....	$\text{H}_2\text{O}$	0	11.0	17.0	10.0	13.0	13.0	9.0
Soil saturated with N/5 $\text{Na}_2\text{SO}_4$ .....	$\text{H}_2\text{O}$	0	6.0†	11.5	5.0	9.0	9.0	5.0

†  $\text{CaCO}_3$  mixed with the soil after first treatment with  $\text{H}_2\text{O}$ .

TABLE 4  
ALKALINITY DEVELOPED BY EXTRACTING SOIL 430\* WITH N/5  $\text{NaNO}_3$ ,  
THEN WITH  $\text{H}_2\text{O}$

Preliminary treatment	Secondary treatment	Order of secondary treatments cc. N/50 HCl per liter (phenolphthalein)						
		1st	2nd	3rd	4th	5th	6th	7th
Soil saturated with N/5 $\text{NaNO}_3$ .....	$\text{H}_2\text{O}$	0	0	0	0	0	0	0
Soil + $\text{CaCO}_3$ saturated with N/5 $\text{NaNO}_3$ .....	$\text{H}_2\text{O}$	0	15.0	27.0	21.5	20.0	18.0	13.3
Soil saturated with N/5 $\text{NaNO}_3$ .....	$\text{H}_2\text{O}$	0	5.0†	17.0	12.0	12.0	11.2	7.3

\* Similar results were obtained with soil 431 except that a greater number of extractions with water was necessary before phenolphthalein alkalinity developed. The amount of alkalinity produced, however, was greater than with soil 430.

†  $\text{CaCO}_3$  mixed with the soil after first treatment with  $\text{H}_2\text{O}$ .

Appreciable amounts of phenolphthalein alkalinity were produced with both soils, only, however, when  $\text{CaCO}_3$  was present. No very marked differences were obtained when the limestone was added to the soil either before or after the treatment with the neutral sodium salt.

Apparently, these data confirm Gedroits' conclusion that  $\text{CaCO}_3$  is essential for the formation of an appreciable amount of  $\text{Na}_2\text{CO}_3$ . It was believed, however, that the experimental technique employed involved conditions so unlike those obtaining in nature, that conclusions drawn from such a study should not be considered final. Some means whereby the water is allowed to percolate through a soil

column was thought to approximate more nearly the natural leaching of a soil. Where this method was employed much more striking and totally different results were obtained.

### *The Genesis of Sodium Carbonate*

In this study 5-kilogram portions of soil No. 430, taken from the Citrus Experiment Station site, and which contained no  $\text{CaCO}_3$ , were placed in large bottles and treated three times with  $12\frac{1}{2}$  liter portions of normal sodium chlorid solution, the supernatant liquid being siphoned off each time. The soil was finally drained on a large Büchner funnel, air dried, and pulverized. It was then placed in 3-inch tubes, without packing, and a constant head of pure water maintained on each soil column. On February 10, 1921, twenty-five hundred grams of air-dried soil was placed in tube 1, and an equal amount of the same soil was placed in tube 2 after adding 2 per cent of precipitated C. P. calcium carbonate and intimately mixing. The water percolated through the soil columns exceedingly slowly, but the volume of percolate from tube 2 was considerably greater than that from tube 1. The first portions of the percolates from both tubes were high in sodium chlorid. The percolate from tube 1 was not alkaline to phenolphthalein at first, while that from tube 2 was distinctly so. This was, however, evidently due to the reaction between calcium carbonate and sodium chlorid and should not be confused with the much greater alkalinity that developed later. In three weeks the percolate from tube 1 manifested alkalinity to phenolphthalein, the amount of which increased rapidly.

TABLE 5  
ALKALINITY DEVELOPED BY EXTRACTING SOIL 430 WITH NORMAL  $\text{NaCl}$  AND  
THEN PERCOLATING WATER THROUGH IT

Date	cc. of percolate obtained	cc. N/10 acid per liter (Phenolph.)	cc. N/10 acid per liter (Methyl orange)	Gm. $\text{Na}_2\text{CO}_3$ per liter	Gm. $\text{Na}_2\text{CO}_3$ total percolate
Soil alone—					
March 12.....	55	2480	5800	26.288	1.446
March 16.....	40	3450	7360	36.57	1.463
Soil + $\text{CaCO}_3$ —					
March 12.....	275	370	1000	3.922	1.078
March 16.....	800	100	500	1.06	0.848

Table 5 shows the extraordinarily high concentrations of sodium carbonate obtained on March 12 and 16. The soil without calcium carbonate yielded a percolate containing phenolphthalein alkalinity equivalent to 3.66 per cent sodium carbonate, while with calcium carbonate the highest concentration was 0.39 per cent sodium carbonate. When the total volumes of the percolates are considered, however, the tubes are seen to have yielded more nearly the same total amounts of sodium carbonate.

The results are considered to indicate: (1) that calcium carbonate is not essential to the formation of sodium carbonate in leaching a salt-saturated soil with water; (2) that important amounts of sodium carbonate may be formed in nature by the percolation of relatively pure water through soils that have been previously saturated with neutral sodium salts.

The production of alkalinity in this manner has not been generally recognized by soil investigators, but these results indicate that this is an important agency in "black-alkali" formation. The conditions necessary for the progress of the reaction are more simple and easily realizable in the state of nature than some of the many possible reactions that have been suggested by previous workers.<sup>1, 5, 6, 7, 31, 37, 41, 43</sup>

Percolation studies using soil saturated with NaCl were repeated several times with similar results. The general course of the percolations was always an initial rapid penetration of the water, with a lowering of the rate usually occurring long before any percolate had passed entirely through the soil column. When the soil was free from  $\text{CaCO}_3$  at the outset, the first portions of the percolates obtained were very concentrated with respect to NaCl and were not alkaline to phenolphthalein. High alkalinity in the percolates usually appeared some days after the first water passed through, although several weeks were frequently required, so greatly was the soil deflocculated. The production of alkalinity usually continued for several days or weeks, and then, if water continued to pass through at all, it began to diminish until in some cases no further phenolphthalein alkalinity was produced.

The rate of percolation was always very slow. With a heavier soil, No. 431, from La Habra, California, water sometimes failed to percolate through the column for a period of several weeks. Only 4 cc. of percolate were obtained from an almost neutral silt loam soil



from Indiana, after it had first been leached with NaCl solution. This small amount of liquid, however, was alkaline to phenolphthalein. An acid Indiana soil, giving a strong test for acidity by Comber's test<sup>8</sup> was first leached with NaCl, then dried and placed in a percolation tube. This soil gave a water extract with pH 5.0 and in the treatment with salt yielded copious amounts of aluminum and manganese in addition to calcium, magnesium, and potassium. Water percolated slowly through this soil and the percolate showed a progressive rise in pH value from 5 to 7.8. Thus, while this distinctly acid soil was not converted into a "black-alkali" soil, it became distinctly alkaline by treatment merely with NaCl and water. It also showed the same tendency to become deflocculated and impermeable to water as that shown by semi-arid soils.

Several attempts were made to increase the permeability of these soils by admixing them with three to four times their weight of sand, broken pottery, and other inert substances, but the dispersion of the colloidal material was so great that little better results were obtained by this means. In certain cases the deflocculated soil was removed from the tubes, air dried, pulverized, and returned to the tubes. Percolation after such treatment was scarcely improved. The physical properties of the soil apparently had been permanently altered by the changes that took place in the initial treatment with sodium chlorid.

In the preceding experiment no provision was made to exclude carbon dioxide and, because of the long time necessary to obtain measurable amounts of percolates, the alkalinity obtained was largely carbonate and bicarbonate alkalinity. To determine whether sodium hydroxide is the alkaline compound resulting directly from the hydrolysis of the sodium "absorbates" another percolation study was made.

Five kilograms of soil 430 was leached with many liters of normal sodium sulfate solution on a Büchner funnel. The soil was finally drained on the funnel by suction, air dried, and pulverized. It was then placed in a tube arranged for percolation in the absence of carbon dioxide and under reduced pressure.

To remove absorbed CO<sub>2</sub> from the dried soil, a stream of air, freed from CO<sub>2</sub> by bubbling through a concentrated solution of sodium hydroxide, was drawn through the soil for eighteen hours. Connection was then made with a bottle of carbon-dioxide-free water, without

breaking the partial vacuum in the apparatus. Water was immediately drawn over on the soil column and allowed to percolate downward by gentle suction. The first drops of percolate were obtained within a few hours and for some time the rate of percolation continued at the rate of about 10 cc. per hour. In two days faint phenolphthalein alkalinity appeared, the amount increasing rapidly, and the percolate became deeply colored. Ultimately it became impossible to draw any further liquid through the soil column.

The final percolate was strongly alkaline, giving a pH-value of 12.85, corresponding to a hydroxyl-ion concentration of  $7.13 \times 10^{-2}$  or a hydrogen-ion concentration of  $0.142 \times 10^{-12}$ . This solution gave a titration figure corresponding to 10,500 cc. of N/10 acid to neutralize 1 liter to phenolphthalein, and 4100 cc. additional acid to neutralize 1 liter to methyl orange. The results, therefore, correspond to the titration of a mixture of hydrate and carbonate or silicate. The data are shown in table 6.

TABLE 6  
PERCOLATES OBTAINED FROM SOIL 430 IN THE ABSENCE OF FREE CARBON DIOXIDE

Date	Character of percolate	Volume of percolate (cc.)	Test for SO <sub>4</sub>	Alkalinity cc. N/10 acid per liter	
				Phenolphthalein	Methyl orange
March 15	Muddy.....	35	Very high	0	45.0
March 15	Clear, straw color.....	10	Very high	0	50.0
March 15	Clear, straw color.....	20	Very high	0	.....
March 16	Clear, straw color.....	15	Very high	0	.....
March 16	Clear, light straw color..	10	Very high	Trace	.....
March 16	Clear, light straw color..	12	Very high	Trace	40.0
March 17	Clear, light straw color..	8	Very high	Trace	40.0
March 17	Muddy, dark color.....	25	.....	.....	.....
March 18	Dark color.....	2	High	350	900.*
March 18	Dark color.....	10	High	2060	3140.†
March 18	Dark color.....	5	High	600	.....
March 19	Very dark, free from suspended matter.....	20	High	10500	14600.‡

\* Carbonate and bicarbonate present.      ‡ Largely hydrate or silicate.  
† Hydrate and carbonate or silicate.

Additional confirmation of the presence of hydrate is indicated by the titrations made upon the dialysates of the final percolate obtained above, as given in table 7. The results of this experiment, therefore,

substantiate the "absorbate" theory, in that sodium hydroxide appears to be the primary source of the alkalinity developed on leaching a sodium-salt-treated soil with pure water.

TABLE 7  
ALKALINITY OF 10 CC. OF PERCOLATE AFTER DIALYSIS  
cc. N/10 acid

	Phenolphthalein	Methyl orange
1st dialysate.....	4.10	5.20
2nd dialysate.....	2.10	3.25
3rd dialysate*.....	Trace	.....

\* The liquid remaining in the dialyzing sack was not alkaline to phenolphthalein, and contained practically no sulphates.

The possibility that the alkalinity may have been produced by sodium silicate has not been excluded. Some of the alkaline extracts were both titrated and analyzed for carbon dioxide and silica, but the amounts available for these purposes were so small that accurate analyses were difficult. In the portions tested no appreciable amounts of silica were found. The percolates obtained from open tubes always contained actual carbonate, but generally in amounts somewhat smaller than that represented by the titration figures. Although sodium silicate may have been one of the initial decomposition products, this probably hydrolyzed immediately, NaOH and silicic acid being formed and the latter either remaining in colloidal solution or being precipitated. In either case the silica would be removed from active chemical action, and NaOH may, therefore, be considered the initial alkaline hydrolytic product of the "absorbate."\* It is the removal of the hydroxyl-ions by the CO<sub>2</sub> of the air that insures the continued decomposition of the "absorbate" and accounts for the progressive accumulation of Na<sub>2</sub>CO<sub>3</sub>.

#### *Development of alkalinity from granitic rocks*

The exchange of bases between salt solutions and the unaltered minerals in rocks has been abundantly confirmed.<sup>9, 11, 21, 25, 32, 42</sup> It is interesting, therefore, to determine whether such a material as

\* Kahlenberg and Lincoln<sup>47</sup> have shown by freezing-point determinations and conductivity measurements that in solutions of the simple alkali silicates, hydrolysis into the hydroxide and colloidal silicic acid is practically complete in concentrations up to about 1/100 normal.

powdered granite will develop alkalinity upon treatment with sodium chlorid and subsequent leaching with water. It was believed that positive data on this point would furnish additional evidence in support of the "absorbate" theory.

For this experiment specimens of unaltered and of weathered granites were secured from Mt. Rubidoux, Riverside. They were ground to pass through a 60-mesh sieve, leached on Büchner funnels with normal sodium chlorid solutions,\* then dried and placed in tubes, as previously described for the soils. Alkaline percolates were obtained as shown in table 8. As a check on the solubility of the granites, pure water and normal sodium chlorid solutions were percolated through columns of untreated granite, but no phenolphthalein alkalinity was produced.†

TABLE 8

ALKALINITY OF PERCOLATES OBTAINED FROM POWDERED GRANITE PREVIOUSLY  
TREATED WITH NaCl

Unaltered Granite

	Vol. of percolate (c.c.)	cc. N/10 acid per liter		Gm. $\text{Na}_2\text{CO}_3$ per liter	Gm. $\text{Na}_2\text{CO}_3$ in total volume obtained
		Phenolph- thalein	Methyl orange		
March 30.....	600	11.5	82.5	0.122	.073
March 31.....	800	12.0	84.0	0.127	.102
April 4.....	800	Trace	65.3	Trace	Trace

Weathered Granite

March 30.....	2000	5.0	52.0	.053	.106
March 31.....	2500	4.5	23.5	.048	.120
April 4.....	2200	2.2	22.0	.023	.051

The results show that appreciable amounts of sodium carbonate may be formed by merely treating powdered granite with NaCl and then percolating water through a column of the material. This occurred both with fresh rock and with somewhat altered rock. The amounts of alkalinity produced were much smaller than with the soil

\* Notable amounts of calcium were found in the sodium chlorid percolates, indicating basic exchange.

† The fact that no phenolphthalein alkalinity was observed in the water percolates of the untreated granites should not be interpreted as meaning that the minerals in the rock are incapable of hydrolysis in pure water, but rather that the conditions of the experiment did not allow this phenomenon to be demonstrated. Portions of the finely ground rock shaken with carbon-dioxide-free water, gave alkaline reactions to phenolphthalein, especially if allowed to evaporate at ordinary temperature exposed to the atmosphere.



derived from these rocks, and the puddling effect, which was a notable feature of the soil, was not so apparent with the ground rock. This might be expected, however, since the replacement of bases in granite by NaCl was not as great as with the soil.

The behavior of these granites indicates that purely inorganic silicates, and probably not organic compounds, are largely concerned in the phenomena observed in the soils, and further, that the silicates involved need not necessarily be greatly altered or secondary minerals.\*

*Development of alkalinity with pure minerals*

The results obtained with the powdered granites suggested a similar study with pure minerals. The work of the several investigators cited has established the fact that replacement reactions between quite stable and only slightly soluble minerals and saline solutions take place, although the extent of this metathesis is generally not great. With the soil studied and with the Rubidoux granites, calcium was the base most abundantly replaced by sodium. Therefore, several types of naturally occurring calcium silicates and calcium aluminum silicates were studied with respect to their behavior with solutions of NaCl. It was believed that in this manner some insight might be gained as to the nature of the compounds that may be involved in similar processes in soils.

Pure, crystalline specimens of the minerals were crushed and pulverized in an agate mortar. The grinding was continued until all of the material passed through a 100-mesh sieve. Solubility studies were made with these materials, employing carbon-dioxide-free water and N/10 NaCl solutions made up from the very carefully purified salt. Two-gram portions of the minerals were shaken with two liters of the solvent for four hours, the suspensions allowed to stand over night, reshaken on the following day for four hours, then filtered and analyzed. The reactions of the resulting solutions and the soluble calcium and silica were determined. The results are shown in table 9.

\* The chemical analyses of the two granites are:

	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	FeO	TiO <sub>2</sub>	MnO	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	Total
Fresh .....	74.69	12.42	0.33	2.25	.18	.12	1.18	.62	4.28	4.08	.04	100.19
Weathered	74.91	12.45	0.10	1.63	.20	.08	1.27	.44	4.29	4.07	.03	100.17

According to W. Harold Tomlinson, the petrographical composition of the fresh rock, which is classified as a quartz-monzonite, is in descending order of abundance: orthoclase, quartz, oligoclase, biotite, hornblende. No secondary minerals were detected by microscopical examination of the thin rock sections.



TABLE 9  
SOLUBILITY OF MINERALS IN WATER AND N/10 NaCl SOLUTION

Mineral	Solubility in water			Solubility in N/10 NaCl		
	Reaction to phenolphthalein	p.p.m. Ca	p.p.m. SiO <sub>2</sub>	Reaction to phenolphthalein	p.p.m. Ca	p.p.m. SiO <sub>2</sub>
Pectolite.....	Distinct rose....	3.4	3.7	Deep rose*.....	4.6*	5.0*
Apophyllite.....	Very deep rose	3.6	3.7	Very deep rose	8.8	9.0
Wollastonite.....	Pink.....	2.8	3.4	Distinct pink..	6.8	7.0
Natrolite.....	Distinct pink..	1.2	0.7	Distinct pink..	6.1	1.3
Thomsonite.....	Faint pink.....	1.5	0.6	Faint pink.....	11.1	1.4
Chabazite.....	Colorless.....	0.7	0.7	Colorless.....	34.0	1.1
Labradorite.....	Colorless.....	0.5	2.7	Colorless.....	4.0	2.4
Anorthite.....	Decided pink..	6.0	1.4	Rose.....	15.7	2.5

\* N/100 NaCl solution used.

Pectolite, apophyllite, and wollastonite are similar in being relatively soluble and easily hydrolyzable in pure water. The solubility of both calcium and silica was distinctly higher than was the case with the other minerals with the exception of anorthite. The concentration of hydroxyl-ions and the total titratable alkalinity were likewise greater. With the use of NaCl, however, the calcium replaced was not great with pectolite, apophyllite, or wollastonite, and an attendant increase in soluble silica was noted in each case.

On the other hand, chabazite and thomsonite, both of very low solubility in pure water, showed pronounced replacement of calcium by NaCl. At the same time, there was no marked increase in the solubility of the silica. With chabazite, 0.069 grams of calcium were found in the two liters of NaCl solution. Since the original two grams of the mineral contained but 0.158 grams of calcium, 43.6 per cent\* of the calcium had been replaced by sodium. It is not believed that such mild treatment as several hours digestion in a dilute saline solution at room temperature could have altered the fundamental structure of the silicate molecule, but rather that the resulting compound was similar structurally to the original calcium aluminum silicate, a portion of the calcium being merely substituted by sodium. Chabazite was the most reactive mineral studied, but thomsonite and anorthite showed considerable reactivity with NaCl.

\* Assuming a composition expressed by the formula  $\text{CaAl}_2\text{Si}_4\text{O}_{12}\cdot 6\text{H}_2\text{O}$ , which was approximately the case, since the mineral actually contained 19.47 per cent combined water as contrasted with the 21.32 per cent required by the formula.

The behavior of these minerals with sodium chlorid is strikingly similar to that of soils 430 and 431. Tests were, therefore, made by first leaching columns of the powdered minerals with solutions of sodium chlorid and then replacing the saline solutions with pure water. The phenomena observed were in some cases strikingly similar to those occurring in soil and corroborate the main assumptions of the "absorbate" theory.

The amount of material available for the percolation studies was limited and variable. In some cases only a few grams of material were used. The technique consisted in the use of small glass tubes in which the mineral powders were supported on a layer of pure quartz sand. Normal solutions of sodium chlorid were first percolated through the columns until the percolates no longer gave definite tests for calcium. Pure water was then poured into the tubes.

In the experiment with thomsonite, fifty grams of material was used. The NaCl solution percolated through this column was not alkaline to phenolphthalein, but immediately upon replacing the saline solution with pure water, a striking alkalinity was apparent in the percolate, quickly followed by a pronounced opalescence and then turbidity. This latter eventually became a very fine suspension, some of which settled out and proved to be pure silica. At the same time the rate of percolation decreased, as with soil, although the deflocculation was not nearly so great. Portions of the percolate were tested, with results as shown in table 10.

TABLE 10  
ALKALINITY PRODUCED BY LEACHING NaCl-TREATED THOMSONITE  
WITH PURE WATER

Percolate	Vol. cc.	cc. N/10 acid required to neutral- ize 1 liter		Phenolphthalein titration calculated as Na <sub>2</sub> CO <sub>3</sub> per liter	Actual Na <sub>2</sub> CO <sub>3</sub> in percolate
		Phenolph.	Methyl orange		
1st.....	45	60	112	0.635 gm.	0.023 gm.
2nd.....	150	40	80	0.424 gm.	0.063 gm.
3rd.....	35	20	56	0.212 gm.	0.007 gm.
4th.....	425	10	34	0.106 gm.	0.044 gm.
Total.....	655	.....	.....	.....	0.137 gm.

Similar results were obtained with chabazite. Only 1.5 grams of this mineral was available for the percolation test, yet this small amount yielded an abundant amount of calcium with the NaCl solution

and gave a pronounced alkalinity upon subsequent leaching with water. Anorthite behaved similarly, while the sample of labradorite tested, although larger in amount, did not yield nearly as much phenolphthalein alkalinity. Wollastonite, pectolite, and apophyllite gave alkaline percolates with sodium chlorid, and the amount of calcium taken into solution was noticeably smaller than with the other minerals. Upon leaching with water the alkalinity of the percolates increased somewhat, but not to the same extent as with thomsonite, chabazite, anorthite, and labradorite. The results are shown in table 11.

TABLE 11  
ALKALINITY PRODUCED BY VARIOUS MINERALS ON LEACHING WITH  
NaCl SOLUTIONS AND H<sub>2</sub>O\*

Mineral	Treatment	
	NaCl cc. N/10 acid per liter	H <sub>2</sub> O after NaCl (phenolphthalein)
Pectolite.....	5.0	8.0
Apophyllite.....	10.0	15.0
Chabazite.....	none	20.0
Wollastonite.....	32.0	53.0
Anorthite.....	none	150.0
Labradorite.....	none	75.0

\* Unequal amounts of the different minerals were used. Therefore the amounts of alkalinity developed should not be regarded as being quantitatively comparable. Labradorite was used in the largest amount, but the quantity of anorthite was much in excess of that of the other minerals except labradorite. The quantity of chabazite used (1.5 gm.) was much less than that of any other mineral.

The results indicate that the calcium of the hydrated calcium aluminum silicates of the thomsonite-chabazite type is the most readily replaced by sodium, and that the resulting silicates, relatively richer in sodium, hydrolyze in pure water, acting then as a primary source of alkalinity. The two plagioclase feldspars studied, anorthite and labradorite, exhibited similar properties and acted in a strictly analogous manner, although the amounts of alkalinity yielded by these minerals, considering the quantities used, were smaller than with the zeolites.

Wollastonite, pectolite, and apophyllite, representing an anhydrous meta-silicate, an anhydrous zeolite-like mineral, and a hydrated alkaline zeolite, respectively, did not react freely with NaCl and,

consequently, having been relatively unsaturated with sodium, did not manifest greatly increased hydrolytic capacity with pure water. - The distinct solubility of unaltered pectolite, apophyllite, anorthite, and wollastonite, in pure water, is, however, significantly greater than that of the other minerals tested, and is sufficient to make such minerals potent sources of alkalinity without the intermediate reaction with solutions of sodium salts. It is proposed to continue the study of pure minerals in an effort to establish the relationship between composition and structure of the silicates and their behavior with salt solutions.

*The concentration of NaCl necessary to form "absorbates"  
in the soil*

It is probable that as the soil silicates become more highly saturated with sodium, they will hydrolyze the more readily upon subsequent removal of the excess of salt. It was decided to determine to what degree the saturation must be carried before alkalinity could be produced.

For this study large quantities of soil 430 were placed on Büchner funnels and leached with NaCl solutions of various concentrations. After the treatment, the soil was air dried, pulverized, and placed in percolation tubes. The percolations proceeded under constant and equal heads of distilled water, as previously described. The results are tabulated in table 12.

TABLE 12  
EFFECT OF CONCENTRATION OF NaCl ON THE FORMATION OF "ABSORBATE"\*

Normality NaCl used	P.P.M.Na.	cc. N/10 acid required to neutralize one liter of percolate								
		June 11	June 13	June 14	June 18	June 21	June 24	July 5	July 19	July 26
.001	23	0	0	0	0	0	0	0	0	0
.01	230	no perc.	no perc.	no perc.	0	5.0	10	20	30	50
0.1	2,300	0	no perc.	Alk.	50	70.0	0†	0†	0†	no perc.
1.0	23,000	no perc.	0	0	130	350	350	420	0†	0†
4.0	92,000	0	0	0	10	120.0	0†	112	40	50

\* Series started June 10.

† Reaction between iron rust and NaCl believed to have resulted in the formation of  $\text{FeCl}_3$  and  $\text{H}^+$  by hydrolysis.

It is clear from the table that a solution of NaCl containing 23 p.p.m. of sodium is not sufficient to produce an appreciable amount of sodium "absorbate" in this soil, but that a concentration of 2300 p.p.m. has a pronounced effect. The action of the normal solution (23,000 p.p.m.) is very notable, as shown in tables 5 and 6. The effect of 4 N NaCl is not easily understood. Its effect in producing alkalinity, as shown here, is considerably less than that of N/1 solution, but whether this was due to the difficulty in removing the large excess of NaCl or to a greater action on the iron rust accidentally introduced into the experiment, or to other causes, is not evident. Theoretically, 4 N NaCl should be more effective than N/1 NaCl, but it is not certain that other complicating factors were absent. This apparent anomaly should be investigated further.

The results of this experiment harmonize with many observations made in the field. Irrigation waters containing small amounts of sodium salts have often been used throughout the west without the production of alkalinity or undesirable physical conditions. With water containing more than 200 parts per million of sodium, however, injurious effects have been observed, which in certain instances, have developed in a relatively brief period. Accumulations of sodium salts in soils are frequently found, sufficient to give solutions of high concentration with rain water. The formation of hydrolyzable "absorbates," therefore, may readily take place in nature. Since the necessary concentration of sodium salts is low, it is possible that sodium carbonate may be formed wherever neutral salts occur. The limitation of the process appears to lie only in the complete saturation and subsequent complete hydrolysis of the silicates involved, but before this stage is reached the physical properties of the soil will become seriously deteriorated.

*The extent to which the excess of NaCl must be removed  
before alkalinity develops*

In the preceding experiments it was necessary to remove a part of the electrolyte from the salt-saturated soil before alkaline percolates were obtained. It has been observed many times in this laboratory, however, that dark-colored percolates and an impervious soil have resulted when only a small fraction of the total salt has been removed. Determination of the equilibria between the sodium of the newly formed "absorbates" and the sodium-ions in the solution surrounding



the solid particles should indicate the extent to which leaching must take place before hydrolysis is possible.

As water percolates downward through a column of soil it may leach out practically all of the salts from the upper layers, thus affording opportunity for hydrolysis and swelling in the upper portion of the column, but under these conditions the exact concentration of salt at the point where hydrolysis begins cannot be determined easily.

As a means of studying this point, soil 430 was first saturated with N/1 NaCl solution, air dried, pulverized, and placed in percolation tubes. Solutions of NaCl of various concentrations, ranging from slightly less than N/1 to N/1000 were then used instead of pure water. It is evident that the concentration of NaCl could not fall below that of the percolant, at any point in the soil. The results are shown in table 13.

TABLE 13

EFFECT OF CONCENTRATION OF NaCl ON THE HYDROLYSIS OF SOIL SATURATED WITH NORMAL NaCl SOLUTION

Percolant	cc. N/10 acid per liter of percolate (Phenolph.)							
	June 11	June 13	June 14	June 18	June 21	June 24	July 5	July 19
H <sub>2</sub> O.....		0	0	130	350	350	420	0
0.01 N NaCl.....		34	60	20	Trace	0	0	0
0.1 N NaCl.....		40	40	8.0	0	0	0	0
0.5 N NaCl.....	Alk.	60	0	0	0	0	0	16
0.8 N NaCl.....	0	0	0	0	0	0		

The data show that the "absorbates" formed by a N/1 NaCl solution may hydrolyze to a certain extent in 0.5 N NaCl solution, but not in a concentration of 0.8 N NaCl. The hydrolysis became more marked as the concentration was reduced to 0.1 N and 0.01 N, but even then the extent of the hydrolysis was much less than with pure water. It would appear, therefore, that the more thorough the leaching the greater will be the formation of sodium carbonate.\*

*The equilibrium between sodium and calcium in  
"absorbate" formation*

To study the equilibrium between sodium and calcium in "absorbate" formation, portions of soil 430 were leached with solutions

\* Preliminary experiments made by E. E. Thomas indicate that prolonged leaching of semi-arid soils with dilute solutions of neutral sodium salts alone may result in the production of distinctly alkaline percolates.

containing a definite amount of NaCl, but variable amounts of CaCl<sub>2</sub>. The soil was then dried and pure water percolated through the soil columns as previously described. The results submitted in table 14 indicate that concentrations of CaCl<sub>2</sub> ranging from 0.001 N to 0.01 N are insufficient to prevent the action of N/1 NaCl; that 0.1 N solution of CaCl<sub>2</sub> effectively prevents it; while 0.001 N CaCl<sub>2</sub> solution was apparently without effect.

TABLE 14  
EFFECT OF THE CONCENTRATION OF CALCIUM ON THE FORMATION OF  
SODIUM “ABSORBATES”

Original solution	cc. N/10 acid required to neutralize 1 liter of percolate (Phenolph.)								
	June 8	June 10	June 11	June 13	June 14	June 18	June 21	June 24	July 5
N/1 NaCl.....	no perc.	no perc.	no perc.	0	0	130	350	350	420
N/1 NaCl 0.001 N CaCl <sub>2</sub> ....	Alk.	134	170	215	300	400	.....	.....	.....
N/1 NaCl 0.01 N CaCl <sub>2</sub> .....	no perc.	no perc.	no perc.	0	0	5	50	100	100
N/1 NaCl 0.1 N CaCl <sub>2</sub> .....	no perc.	no perc.	no perc.	0	0	0	0	0	0

*The effect of calcium ions upon the hydrolysis of sodium “absorbates”*

For this study soil 430 was saturated with N/1 sodium chlorid as before, but instead of using pure water as the percolant, solutions of CaCl<sub>2</sub> of varying concentrations were employed. The data are shown in table 15.

TABLE 15  
EFFECT OF CaCl<sub>2</sub> ON THE HYDROLYSIS OF SODIUM “ABSORBATES”

Percolant	cc. N/10 acid required to neutralize 1 liter of percolate (Phenolph.)								
	June 8	June 10	June 11	June 13	June 14	June 18	June 21	June 24	July 5
H <sub>2</sub> O.....	no perc.	no perc.	no perc.	0	0	130	350	350	420
N/1000 CaCl <sub>2</sub> .....	Alk.	0	17.5	240	180	100*	180*	230	.....
N/100 CaCl <sub>2</sub> .....	0	0	7.5	140	250	270*	210*	325	.....
N/10 CaCl <sub>2</sub> .....	0	0*	0†	0†	0†	0†	0†	0†	.....

\* Dark-colored percolate.

† Solution high in Ca.

The results show that a solution N/10 with respect to calcium (2000 p.p.m.) prevents the hydrolysis of sodium "absorbates" formed in this soil by N/1 sodium chlorid; but that N/1000 and N/100 calcium solutions are little more effective than pure water. These results indicate that in reclaiming soils which are highly saturated with sodium, the presence of considerable calcium in the irrigation water will aid in preventing their deterioration into alkaline lands.

The action of calcium-ions in preventing the hydrolysis of sodium "absorbates" is probably complex. First, calcium replaces sodium from the silicates forming calcium "absorbates." Second, calcium-ions exert a coagulating effect upon the colloids and thereby prevent their solution and hydrolysis. Third, any carbonate alkalinity produced by the hydrolysis of sodium "absorbates" would be precipitated as calcium carbonate.

#### *The development of alkalinity in natural saline soils*

The conversion of natural saline lands into alkaline lands is reported to have been of great historical interest. Gedroits explains the relationship between these two types of soil and emphasizes that the one must inevitably result from the other, unless calcium salts are used in conjunction with leaching in the reclamation of such lands. Dominici cites instances of the deterioration of vast areas of originally fertile soil, suggesting that they first became saline, then alkaline, and finally impoverished of all fine material by erosion, with resultant sterility. Laboratory confirmation of this view has not been reported, however.

A fine sandy loam soil, No. 887, was used for this purpose. This soil, taken from the Kearney Vineyard, Fresno County, California, contained slightly less than 1.0 per cent total soluble salts, but a 1:5 water extract gave no phenolphthalein alkalinity. A column of the untreated soil was subjected to percolation under a constant head of pure water. The first portions of the percolate were not alkaline to phenolphthalein, just as with the soils artificially treated with sodium chlorid. However, alkalinity soon developed in the percolate. The final percolate required 20 cc. N/10 acid to neutralize 1 liter to phenolphthalein; and 160 cc. for the methyl orange titration. The soil was finally removed from the percolator and extracted with five times its weight of carbon-dioxide-free water. The filtrate, after

passing through a Pasteur-Chamberland tube, gave a faint but noticeable alkaline reaction to phenolphthalein.

This experiment, therefore, affords an example of the conversion of a saline, non-alkaline soil into an alkaline soil, manifesting the physical characteristics of a "black-alkali" soil, containing residual sodium carbonate and yielding a distinctly alkaline percolate.

An interesting confirmation of this point was noted in unpublished experiments by A. R. C. Haas. He grew young orange trees in tanks of soil from the Citrus Experiment Station farm. The trees were irrigated for a time with dilute solutions of sodium chlorid. Later, an attempt was made to remove the excess of salt by leaching with distilled water. The drainage water was found to be distinctly alkaline to phenolphthalein. Continued leaching with distilled water ultimately gave percolates free from phenolphthalein alkalinity. Portions of the soil remaining in the tanks were later extracted with water in the usual 1:5 ratio and extracts with pH-values of 7.0 were obtained. In our work it has been noted that soils yielding very markedly alkaline percolates do not always give alkaline extracts when shaken with an excess of water. How a soil can yield a percolate containing 3 per cent sodium carbonate, for instance, and yet contain no determinable amount of residual alkalinity, is not clear.

#### GENERAL DISCUSSION

Previously two different explanations have been given regarding the origin of sodium carbonate in arid regions. One of these traces it directly to the weathering of igneous and metamorphic rocks; the other to the interaction of neutral sodium salts and calcium carbonate. According to the former view the soluble carbonates brought into solution by the action of meteoric waters tend to accumulate in places where the drainage and other conditions are favorable for their deposition. According to the latter, sodium salts reacting with calcium carbonate give rise to sodium carbonate and a soluble calcium salt. The fact that this latter reaction is capable of taking place has been known since the time of Berthollet<sup>2</sup> and has been periodically investigated down to the present time. Despite the experimental work the kinetics of the reaction have not been thoroughly established. The difficulty seems to lie in the necessity for the separation of the products of the reaction.



A third origin of sodium carbonate, discussed in this paper, has not been generally recognized. This, as set forth above, consists in the more or less complete saturation of the soil by neutral sodium salts, and in the subsequent hydrolysis of the sodium-silicate compounds thus formed. The conditions most favorable for this process are thorough and complete saturation of the soil with sodium and subsequent leaching. The extent to which the process may take place depends upon the nature of the soil, those containing much clay, silt, and abundant quantities of altered and secondary minerals, probably being the more reactive. Ideal conditions for this reaction are probably the exception in nature; but the above experiments show that small but not totally negligible amounts of  $\text{Na}_2\text{CO}_3$  may be formed under less favorable conditions. Where the conditions are decidedly favorable, large amounts of soluble alkaline compounds may eventually be carried down into the ground water or transported by seepage.

This reaction has been shown to be a consequence of basic exchanges between soil silicates and saline solutions. By essentially chemical processes, probably due to differences in their respective electrolytic solution potentials, sodium has been shown to replace a part of the calcium from the soil silicates readily and rapidly. When the concentration of sodium in solution is high and the calcium set free can be readily removed, a relatively high degree of saturation with sodium may occur. The physical properties of the new sodium-silicate combinations are different from those of the original calcium-silicate compounds, and are so pronounced as to alter profoundly the characteristics of the entire soil mass.

The sodium "absorbates," so-called, are much less stable than the corresponding calcium compounds. They are probably colloidal in nature, but this has not been established and is not a necessary assumption for an explanation of their behavior. Their important property is that of slowly hydrolyzing in the absence of strong electrolytes. Hydrolytic equilibrium is not rapidly established in nature because the hydroxyl-ions resulting from hydrolysis are readily removed by the  $\text{CO}_2$  in the soil atmosphere. This favors the decomposition of the "absorbates" and the development of alkalinity. Calcium carbonate may also favor the removal of the hydroxyl-ions.

The other product of the hydrolysis, the silicate complex, shows a tendency to be present in a colloidal state. This, as is well recog-



nized, may be an important factor favoring slow hydrolysis. Furthermore, reactions between the soluble hydrolytic products and the colloid probably favor a high degree of dispersion and account for the marked change in the physical properties of the soil. These striking changes in the physical properties of the soils were always observed associated or coincident with the development of alkalinity, and constitute one of the most important aspects of the phenomenon. The characteristic hardness and impermeability of many alkaline soils may be ascribed to the presence of sodium "absorbates," or the products of their hydrolysis.

Lessons may be drawn from these studies as to the proper treatment of saline soils. Leaching alone may be expected to lower the concentration of soluble salts, but in some cases at least, a deterioration of the land may be expected, partially to offset this advantage. In such cases some means must be provided to prevent the hydrolysis of the sodium "absorbates" with their attendant deflocculation.

Any means of preventing hydrolysis during and after the removal of the soluble salts, such as the addition of soluble aluminum, ferrous and ferric salts, acids and organic matter, may be expected to be beneficial. There may be some objection to the use of some of these substances, however, since the soil may be left in an unfavorable condition for crop growth. Moreover, none of these materials can correct the fundamental mischief caused by the original sodium salts, i.e., they cannot restore the calcium to its original position in the active soil silicates. The importance of calcium in the silicate-complexes of the soil in maintaining a desirable physical and chemical medium for plant growth can hardly be over-emphasized. Soluble calcium salts should not, therefore, be ignored in the practical reclamation of saline lands.

#### SUMMARY

(1) When semi-arid soils are brought to equilibrium with a sodium salt the combined calcium, magnesium, and potassium passing into solution are chemically equivalent to the sodium fixed, and the total of these ions is an exponential function of the concentration of the sodium salt remaining in solution. It was found that the sodium fixed is an exponential function of the concentration of the sodium salt remaining in solution at equilibrium.

(2) These relationships conform to the general type of Freundlich's adsorption curves and equations. The reactions are considered to be primarily chemical in nature.

(3) Upon treatment with a sodium salt, the soil undergoes a change reciprocal to that occurring in the solution. While the solution becomes enriched with calcium, the soil becomes impoverished of that element. The solid particles, therefore, become relatively richer in sodium.

(4) The new sodium-silicate complexes formed by the action of sodium salts are less stable, more soluble, and more easily hydrolyzable than the corresponding calcium complexes. The properties of these sodium compounds are so pronounced as to modify profoundly the physical properties of the entire soil mass.

(5) The effects of saturating a soil with sodium are most pronounced upon subsequent leaching with relatively pure water. The soil then swells, becomes impervious and yields a dark-colored and frequently strongly alkaline percolate. These reactions are considered to be important in the formation of sodium carbonate in nature.

(6) The formation of alkalinity is dependent upon the tendency of the sodium-silicate compounds to hydrolyze in the absence of too strong concentrations of electrolytes, with the resultant formation of sodium hydroxide. The NaOH is readily converted into  $\text{Na}_2\text{CO}_3$  by the  $\text{CO}_2$  of the soil solution.

(7) Some of the products of the hydrolysis have a tendency to assume the colloidal state, which, probably accentuated and maintained by the hydroxyl-ions, accounts for the deflocculated condition of the soil. Calcium carbonate was shown not to be essential for the formation of  $\text{Na}_2\text{CO}_3$  by this reaction.

(8) Certain soils from humid regions, one of which was distinctly acid, reacted similarly but not as markedly as semi-arid soils.

(9) Alkalinity was also developed by percolating water through columns of granites and several pure mineral silicates which had previously been treated with sodium chlorid.

(10) With the soil studied N/100 solution of sodium chlorid was found to be of sufficient concentration to form hydrolyzable compounds; N/10 and N/1 solutions were still more potent in this respect, while N/1000 solutions had no apparent effect.

(11) It was found that the concentration of the saturating solution of sodium chlorid must be reduced about one-half before alkalinity develops, but that lowering the concentration still further greatly increases the alkalinity. The greatest amount of alkalinity was obtained when the sodium-saturated soil was leached with pure water.

(12) Soluble calcium salts, if present in sufficient concentration, will prevent the formation of hydrolyzable sodium compounds in the soil. With a mixed solution of normal sodium chlorid the addition of  $\text{CaCl}_2$  equivalent to N/1000 had no effect; N/100  $\text{CaCl}_2$  reduced the amount, while N/10  $\text{CaCl}_2$  completely prevented the reaction.

(13) Calcium-ions tend to prevent the hydrolysis of sodium absorption compounds once they have been formed. N/10  $\text{CaCl}_2$  solution completely prevented the hydrolysis in soil 430, while N/100 and N/1000  $\text{CaCl}_2$  solution were little more effective than pure water.

(14) An example is given of the conversion of a natural saline soil into an alkaline soil, and of the development of alkalinity in a soil by mild treatment with sodium chlorid and subsequent leaching with pure water.

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SITION OF YOUNG ORANGE TREES

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---

Citrus trees grown with artificial irrigation frequently show certain pathological conditions which are believed to be due to an excess of sodium salts. Little is known regarding the effect of an excess or a deficiency of one or more ions upon the absorption of other ions by citrus trees, or the effect of non-essential salts on the absorption of essential ions. We do not know as yet whether citrus trees have the power of regulating their composition to any marked degree, or whether a given variation in the composition of the tree necessarily reflects a corresponding variation in the nutrient medium. Before any explanations of disturbances in the nutrition of citrus trees can be safely accepted, it is essential that such disturbances be produced in experiments, the factors of which are capable of being scientifically analyzed.

In order to throw some light upon this problem, orange trees were grown in pure sand in which the effect of sodium chlorid was studied in the presence and absence of calcium. Such experiments should prove to be of interest, since Kelley and Cummins<sup>1</sup> have shown mottled leaves to be deficient in calcium. The results of experiments in which the effect of sodium chlorid was studied in soil cultures will be presented in a subsequent paper.

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\* Paper No. 101, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

Valencia orange trees (*Citrus sinensis*) budded on sour-orange (*Citrus aurantium*) root-stocks were used in the experiments. The details of the method of growing the trees have been previously described.<sup>2</sup>

The stock solutions used in the present studies were as follows:

*Solution A*

KNO <sub>3</sub> .....	1200 gm.
MgSO <sub>4</sub> + 7H <sub>2</sub> O .....	1800 gm.
NaCl .....	55 gm.
H <sub>2</sub> O to give volume of.....	18 liters

*Solution C*

KH <sub>2</sub> PO <sub>4</sub> .....	900 gm.
H <sub>2</sub> O to give volume of.....	18 liters

*Solution D*

KNO <sub>3</sub> .....	2226.3 gm.
H <sub>2</sub> O to give volume of.....	18 liters

*Solution E*

NaCl .....	1800 gm.
H <sub>2</sub> O to give volume of.....	18 liters

*Solution F*

CaCl <sub>2</sub> + 2H <sub>2</sub> O .....	1613.3 gm.
H <sub>2</sub> O to give volume of.....	18 liters

*Solution G*

MnSO <sub>4</sub> + 4H <sub>2</sub> O .....	0.406 gm.
H <sub>2</sub> O to give volume of.....	2 liters

The trees in cans 42-46, inclusive, received Hoagland's nutrient solution<sup>2</sup> in which potassium replaced calcium. This solution was prepared by using the following amounts of the stock solutions: 55 c.c. of solution A, 30 c.c. of solution C, 65 c.c. of solution D, and 20 c.c. of solution G, increased to 10 liters with distilled water.

Here we have substituted potassium nitrate for calcium nitrate in the solution devised by Hoagland and by so doing have greatly increased the concentration of potassium. Trelease<sup>3</sup> found that wheat plants are not very sensitive to potassium chlorid in the culture solution even when this salt constituted 90 per cent of the osmotic value of the solution, any retarding effects being attributed rather to the low concentration of the other three salts.

The trees grown in cans 6-11, inclusive received the same culture solution as those in cans 42-46 except that 1000 p.p.m. sodium chlorid was added by including 100 c.c. of stock solution E in making up the volume of the culture solution to 10 liters.

The trees grown in cans 12-17, inclusive, received the same culture solution as those in cans 6-11, plus calcium equivalent to that of Hoagland's solution added in the form of calcium chlorid; 65 c.c. of stock solution F were added in making up each 10 liters of culture solution given cans 12-17. The concentration of the various ions in the culture solutions is given in table 1.

TABLE 1  
PARTS PER MILLION IN CULTURE SOLUTIONS

	Cans 42-46	Cans 6-11	Cans 12-17
Fe.....	1	1	1
Mn.....	0.1	0.1	0.1
Ca.....	.....	.....	159
Mg.....	54	54	54
K.....	496	496	496
Na.....	7	400	400
PO <sub>4</sub> .....	105	105	105
SO <sub>4</sub> .....	216	216	216
Cl.....	10	617	898
NO <sub>3</sub> .....	718	718	718
Total concentration.....	1607.1	2607.1	3047.1

Prior to each addition of the culture solution to the trees, carbon-treated distilled water was added to each can and allowed to percolate through the sand in order to remove as much as possible of the old solution, and then fresh culture solution was added. As a rule, ferric tartrate was added (5 to 10 p.p.m.) to the distilled water and culture solutions just as they were ready to be added to the cans in order to insure a plentiful supply of iron. The culture solutions, which were distinctly acid in their reaction when first made, become more nearly neutral after standing in contact with the silica sand (Shive<sup>4</sup>). The change in reaction was more pronounced in the solution which percolated through the sand in which trees were growing than in the sand alone, as shown in table 2. The reaction values of the sap of mature leaves in the different series in table 2 show no appreciable differences, which fact may be due to the large amounts of buffer substances which the leaves contain. No determination of total acidity of leaf sap were made, although it has been reported<sup>5</sup> that increased absorption of chlorin by buckwheat is accompanied by

increased acidity. It may be of interest to mention in this connection that Wilson<sup>6</sup> has found that the addition of sodium chlorid to an acid solution results in an increase in H-ion concentration, whereas when it is added to an alkaline solution an increase in the OH-ion concentration results.

TABLE 2  
REACTION VALUES OF THE CULTURE SOLUTIONS AND OF LEAF SAP

Series	Initial pH of culture solution	pH of culture solution after 4 weeks			pH of sap of mature leaves
		Without sand	In contact with		
			Sand	Sand and tree roots	
42-46	5.4	5.6	6.4	7.0	5.95
6-11	5.2	5.4	6.5	6.9	5.88
12-17	5.2	5.2	6.0	7.0	5.88

TABLE 3  
OSMOTIC PRESSURE OF CULTURE SOLUTIONS AND OF SAP OF MATURE ORANGE LEAVES

Series	Osmotic pressure	
	Culture solution	Leaf sap
	(atm.)	(atm.)
42-46.....	0.708	22.53
6-11.....	1.459	22.59
12-17.....	1.568	21.26

Table 3 shows that differences in the initial osmotic pressure of the culture solutions were not reflected in the concentration of the leaf sap.

Examinations of the drainage water obtained from each of the series were made by A. B. Cummins. It was found that the percolates contained abundant amounts of  $\text{PO}_4$  and  $\text{NO}_3$  in each case except where Ca was present in amounts sufficient to precipitate most of the  $\text{PO}_4$  added.

The experiments indicate that pruning the tree severely at the time of planting in the culture may hasten the appearance of specific effects of the treatment. Moreover, by observing the trees day by day, we may perchance observe transient effects due to the lack of specific ions which might not be seen in any one of a number of given salt proportions.

There was marked uniformity in the growth of the trees until the first cycle was completed. This no doubt was due to the residuum of ions within the trunk and root. But when the second growth cycle began, about three months later, it soon became evident that a nutritional disturbance had been set up in both of the calcium-free series (cans 6-11 and 42-46). Abscission of leaves took place in these two series with such ease that a gentle wind caused many leaves to fall. Before these leaves fell they were marked with characteristic light-colored spots. Plate 1, figure 1, shows the appearance of leaves taken from tree 43 in December, 1920, seven months after the experiment was begun. The mid-rib and veins turned light yellow; then the entire leaf became yellow and fell. Abscission may take place during any of these stages. It is interesting to note also that many of the leaves showed fading out of the chlorophyll as illustrated in the leaf to the extreme right in plate 1, figure 1.

After the leaves in the two series fell, the buds soon produced an abundance of new leaflets. Very few of these leaflets reached maturity. The young leaves shown in plate 1, figure 2, were photographed in April, 1921, and indicate that the leaves may show this peppering of brown dead spots at any stage in their development. These young leaves soon fell off; the lids of the cans were frequently covered with the fallen leaves in various stages of development. Table 3 indicates that we are not dealing here with an osmotic effect of the culture solution because trees 12-17, which received a more concentrated solution, did not lose their young leaves in this manner. The sand in all cases was kept well irrigated with distilled water followed by culture solution. The effects produced were therefore without doubt due to the extreme calcium deficiency of the culture solution. Where these young leaves are being shed continually and new leaves are being produced, there is a tendency for many of the young leaves to become pale yellow when only partly grown, and later to fall from the tree (pl. 1, fig. 2).

When calcium is very deficient, not only the young leaves but even the young shoot tips may begin to die back. In such cases they usually dies back to the beginning of that particular growth cycle. Plate 2, figure 1, shows young shoot tips suffering from extreme calcium deficiency.

In series 6-11 and 42-46 the uppermost shoots died first, while the tips of the lowermost shoots retained their leaves the longest.



Plate 2, figure 2, shows the tips of upper shoots which had lost their leaves and on which much of the subsequent growth was dead.

As time went on and the effects of calcium deficiency became more pronounced, each crop of new leaves was shorter lived than the preceding. In many cases the small pale green leaves fell off before they reached a length of two centimeters. The repeated defoliation and development of new growth led to the condition known as "multiple buds," in which each lateral bud is surrounded by a number of accessory buds. In such cases many short shoots were formed in a manner which is quite typical of mottled trees in the field. Gradually the trees died from the top of the trunk downward. The few leaves remaining on these calcium-deficient trees were curled somewhat like lemon leaves, in contrast to the normal method of folding inward as shown to the extreme right in plate 3, figure 1.

The trees in cans 6-11 and 42-46 were in such poor condition by September, 1921, that it seemed best to remove them from the cans. The new growth they had produced was very short-lived. Trees 13 and 16 were removed at the same time to serve for comparison. It will be recalled that series 12-17 to which they belonged received 1000 p.p.m. of NaCl in addition to Hoagland's solution in which the full amount of calcium was added in the form of calcium chlorid. Trees 12, 14, 15, and 17 were left in the cans until February, 1922, in order to note whether any marked changes would occur upon the advent of cold weather. No noteworthy changes occurred up to the time the trees were removed in February, 1922, except the loss of some of the older leaves. Since it has been shown<sup>1</sup> that the ash constituents of citrus leaves may vary considerably according to their age, the materials obtained from trees 12, 14, 15, and 17 were not analyzed with the other trees of their series which were removed at an earlier date.

In order to eliminate unnecessary repetition of figures, a single photograph of two or three trees in each series is presented as representing the condition typical of the trees in August, 1921.

Plate 3, figure 2, and plate 4, figures 1 and 2, show the appearance of the trees just prior to being removed from the cans. Many of the young shoots and the upper portion of the trunk in trees 6-11 and 42-46 were dead, or nearly so. There was a distinct tendency for some of the leaves to mottle in both these series as well as in

another series which differed from 6-11 in that sodium sulfate was used instead of sodium chlorid. This tendency to mottle will be discussed later. Many of the rootlets in series 6-11 and 42-46 were dead or had become slimy, the cortex readily sloughing off from the central vascular strand in a manner characteristic of citrus roots grown in the absence of calcium (Reed and Haas<sup>7</sup>). In contrast with this condition, trees 13 and 16 showed no dead shoots or rootlets except where the basal portion of the root system came in contact with accumulated drainage water.

Plate 5, figures 1 and 2, show the effect of calcium deficiency upon the growth of citrus roots. Trees 6-11 suffered less from the effects of the sodium chlorid than from the extreme calcium deficiency of the culture solution, since trees 42-46 which received the same solution as trees 6-11 minus the 1000 p.p.m. NaCl showed quite as severe injury.

The leaves of trees in series 12-17 frequently developed dead areas following temporary wilting, and in severe cases these leaves later fell from the trees.

Table 4 gives data which show the salient facts relating to the growth of each of the trees under discussion. There appears to be less variability in the trees in series 42-46 than in trees of the other series.

The table, as well as the plates (pl. 3, fig. 2, and pl. 4, figs. 1 and 2), show the superiority of series 12-17 over the others. This is evident whether we consider the total weight of material produced or the number of leaves and fruits they bore. The ratio of water transpired to dry weight of tree is lowest for series 12-17.

The various portions of trees 6-11, 42-46, 13, and 16 were dried, ground, and prepared for analysis as previously described.<sup>2</sup> As some of the tree samples were insufficient in amount for a complete analysis, composite samples were made for series 6-11, series 42-46, and trees 13 and 16 as follows: Equal amounts of powdered leaf material of each tree were composited as a sample for analysis. Similar samples were composited for the shoots, trunks, roots, and rootlets. In this manner each tree of a given series had an equal representation in the composite sample to be analyzed.

The composite samples were subjected to analysis by the same methods previously used.<sup>2</sup> The averages of closely agreeing duplicate

TABLE 4  
NUMBER OF LEAVES, DRY WEIGHT OF VARIOUS PORTIONS OF ORANGE TREES, AND WATER REQUIREMENT

No. of cans	No. of leaves on tree	Dry weight (60°-65° C) in grams						Total culture solution added Liters	Total distilled water added Liters	Total drainage water Liters	Transpiration Liters	Water requirement
		Leaves	Shoots	Trunk	Root	Rootlets	Oranges					
42.....	59	20.02	20.4	99	102	20.4	.....	262.0	48	135	119.5	0.456
43.....	68	9.2	17	87	103	12.4	.....	228.6	46	135	77.5	0.339
44.....	75	14.2	31.5	67	132	29	.....	273.7	51	127	102.5	0.374
45.....	262	24	7.5	72	119	14	.....	236.5	49	163	46.5	0.196
46.....	86	27.4	27.5	100.5	117	18.5	.....	290.9	46	140	83.5	0.287
Average.....	110	19	20.8	85.1	115	18.9	.....	258.3	48	140	85.9	0.332
6.....	61	15	13.4	70.5	53	14.2	.....	166.1	49	143	78	0.469
7.....	66	11	16.5	118	104	14	.....	263.5	55	139	88	0.334
8.....	152	18	16	107	76	15	.....	232	52	147	77	0.332
9.....	94	23	18	88	85.5	16	.....	230.5	49	140	81	0.351
10.....	47	3.7	6	75	67	2	.....	153.7	46	169	49	0.319
11.....	163	23	7	92	88	11.5	.....	221.5	52	153	71	0.320
Average.....	97	15.6	12.8	91.7	78.9	12.1	.....	211.2	50	148	74	0.350
12.....	419	65	24	82	106.5	39.5	25.5	342.5	69	194	50	0.146
13.....	475	97.5	33	127.5	135	39.5	10	442.5	68	138	118	0.266
14.....	312	105.5	64.5	128	164.5	98	.....	560.5	113	159	166	0.296
15.....	592	100	47.5	114	128	89	.....	478.5	107	171	144	0.300
16.....	522	92	37.5	81	77	52.5	36	377.0	70	120	134	0.355
17.....	291	73	49	159	168	84.5	.....	533.5	113	174	145	0.272
Average of trees, 13 and 16.....	498	95.2	35.2	104.2	106	46	23	410	69	129	126	0.307
Average (all trees of series 12-17).....	435	89	42.6	115.2	129.8	67.2	11.9	455.9	90	159	126	0.276

determinations are given in tables 5, 6, 7, 8, and 9. The results have been calculated both as percentage of dry matter and as percentage of ash.

TABLE 5  
ANALYSES OF LEAVES

	Expressed as percentage of dry matter			Expressed as percentage of ash		
	Trees 6-11	Trees 13 and 16	Trees 42-46	Trees 6-11	Trees 13 and 16	Trees 42-46
Total N.....	3.22	2.79	2.95			
Ash.....	14.85	16.82	15.75			
K.....	7.43	6.43	7.88	50.07	38.25	50.04
Na.....	0.09	0.27		0.60	1.61	
Ca.....	0.14	1.48	0.20	0.91	8.77	1.28
Mg.....	0.23	0.19	0.18	1.57	1.15	1.16
Cl.....	0.25	0.80	0.02	1.71	4.73	0.10

TABLE 6  
ANALYSES OF SHOOTS

	Expressed as percentage of dry matter			Expressed as percentage of ash		
	Trees 6-11	Trees 13 and 16	Trees 42-46	Trees 6-11	Trees 13 and 16	Trees 42-46
Total N.....	1.84	1.34	1.65			
Ash.....	7.92	7.26	7.84			
K.....	3.44	1.98	3.52	43.48	27.23	44.86
Na.....	0.11	0.08	0.03	1.43	1.14	0.38
Ca.....	0.28	1.17	0.23	3.48	16.07	2.98
Mg.....	0.15	0.17	0.14	1.93	2.36	1.79
Cl.....	0.44	0.41	trace	5.55	5.64	trace

The percentage of total nitrogen was usually greatest in the dry matter of the leaves, and next greatest in the rootlets. The percentage of nitrogen in the several parts of trees 13 and 16 was less than that in corresponding parts of other trees. The percentage decreases as we pass from the leaves to the shoots and again from the shoots to the trunk and root. The percentage of total ash in each series decreases very markedly as we pass from the leaves to the root but that of the rootlets approximates the values obtained for the shoots.

TABLE 7  
ANALYSES OF TRUNK

	Expressed as percentage of dry matter			Expressed as percentage of ash		
	Trees 6-11	Trees 13 and 16	Trees 42-46	Trees 6-11	Trees 13 and 16	Trees 42-46
Total N.....	0.82	0.61	0.83	.....	.....	.....
Ash.....	3.51	3.07	3.73	.....	.....	.....
K.....	0.49	0.61	0.99	14.05	19.92	26.65
Na.....	0.29	0.09	0.04	8.38	2.82	1.19
Ca.....	0.66	0.63	0.62	18.70	20.65	16.50
Mg.....	0.09	0.05	0.10	2.46	1.60	2.81
Cl.....	0.08	0.08	trace	2.15	2.46	trace

TABLE 8  
ANALYSES OF ROOT

	Expressed as percentage of dry matter			Expressed as percentage of ash		
	Trees 6-11	Trees 13 and 16	Trees 42-46	Trees 6-11	Trees 13 and 16	Trees 42-46
Total N.....	0.81	0.77	0.75	.....	.....	.....
Ash.....	2.90	2.77	3.13	.....	.....	.....
K.....	0.28	0.40	0.75	9.66	14.55	24.11
Na.....	0.40	0.21	0.16	13.90	7.73	5.12
Ca.....	0.42	0.50	0.42	14.60	17.94	13.49
Mg.....	0.08	0.04	0.10	2.80	1.56	3.34
Cl.....	0.08	0.09	trace	2.84	3.34	trace

TABLE 9  
ANALYSES OF ROOTLETS CALCULATED TO A SILICA-FREE BASIS

	Expressed as percentage of dry matter			Expressed as percentage of ash		
	Trees 6-11	Trees 13 and 16	Trees 42-46	Trees 6-11	Trees 13 and 16	Trees 42-46
Total N.....	2.21	1.23	2.18	.....	.....	.....
Ash.....	8.15	12.00	7.96	.....	.....	.....
K.....	2.22	2.32	2.92	27.27	19.36	36.71
Na.....	0.79	0.64	0.06	9.75	5.30	0.78
Ca.....	0.29	2.00	0.36	3.56	16.64	4.55
Mg.....	0.26	0.25	0.47	3.25	2.10	5.95
Cl.....	1.08	1.02	0.12	13.21	8.54	1.55



We may now examine the sodium (Na) values obtained in the analyses. Kelley and Cummins<sup>1</sup> have found the percentage of sodium (Na) in the ash of Valencia orange leaves to vary from 1.35 per cent when one week old, to 0.42 per cent when three or more years old. The results obtained by the writers<sup>2</sup> for sodium (Na) in the ash of the leaves from trees grown in sand receiving Hoagland's solution agree well with the results reported for leaves of trees grown in the field. One might ask whether the low percentage content of sodium (Na) in the case of the sand cultures was due to the low sodium content of the medium. The present experiments indicate that such was not the case because when the culture solution contained as much

TABLE 10

TOTAL AMOUNT (IN GRAMS) OF SODIUM AND CHLORIN FOUND IN VARIOUS PARTS  
OF AVERAGE TREE

Culture solution contained	400 p.p.m. Na, no Ca		400 p.p.m. Na, 159 p.p.m. Ca and 898 p.p.m. Cl		7 p.p.m. Na, no Ca, and 10 p.p.m. Cl.	
	Trees 6-11		Trees 13 and 16		Trees 42-46	
	Na	Cl	Na	Cl	Na	Cl
Leaves.....	0.0140	0.0406	0.2570	0.7521	trace	0.0038
Shoots.....	0.0141	0.0563	0.0282	0.1443	0.0062	trace
Trunk.....	0.2751	0.0734	0.0938	0.0834	0.0426	trace
Root.....	0.3235	0.0710	0.2332	0.0954	0.1955	trace
Rootlets.....	0.0968	0.1307	0.2944	0.4738	0.0132	0.0246
Total per average tree.....	0.7235	0.3720	0.9066	1.5490	0.2575	0.0284

Culture solution contained	7 p.p.m. Na, 159 p.p.m. Ca, and 10 p.p.m. Cl.		7 p.p.m. Na, 159 p.p.m. Ca, and 10 p.p.m. Cl.		(See text)	
	Tree 1		Tree 2		Tree 85	
	Na	Cl	Na	Cl	Na	Cl
Leaves.....	0.3082	0.0948	0.1607	0.0383	0.3294	0.0412
Shoots.....	0.1593	0.0123	0.0559	0.0021	0.0768	0.0096
Trunk.....	0.3492	trace	0.3024	0.0013	0.1546	0.0084
Root.....	0.2968	0.0064	0.0640	0.0038	0.0945	0.0216
Rootlets.....	0.1632	0.1632	0.0513	0.0741	undetermined	
Total per average tree.....	1.2767	0.2767	0.6343	0.1196	Not including rootlets 0.6553 0.0808	

as 1000 p.p.m. of sodium chlorid, the percentage of sodium (Na) in the leaf ash was still as low as 1.61 per cent or less, while in the shoots it may not have increased much above 1.4 per cent of the ash. The analysis of the leaf ash is most important but cannot be expected to represent completely the composition of a citrus tree. In experiments previously reported<sup>2</sup> in which Hoagland's solution was added to sand and to soil, it was found that sodium (Na) made up an appreciable percentage of the ash of the trunk and root, and to a smaller extent that of the rootlets. Although the percentage of sodium (Na) in the ash of the trunk and root may be considerable, the total amount of this ion actually present in the trunk may be considerably less than in the leaves, due to the larger total dry weight of the leaves. Markwort<sup>8</sup> has reported for sugar beets that sodium applied to the soil migrates almost completely to the leaves and thus reduces their potassium requirement to such an extent that a larger amount of potassium is available for other portions of the plant. The data thus far available have not indicated that an excess of sodium salts tends to reduce the percentage of potassium in the ash of citrus leaves.

The total weights of sodium (Na) and chlorin (Cl) present in the dry matter of trees which received various amounts of NaCl, with or without Ca, are given in table 10.

Table 10 shows that the total amount of sodium (Na) in the tree greatly exceeds that of the chlorin present, except in trees 13 and 16. The exception noted for trees 13 and 16 may be due to the fact that their culture solution contained 281 p.p.m. more Cl than that applied to trees 6-11, which received equivalent amounts of Na and Cl. The small amount of sodium and chlorin reported for the leaves of trees 42-46 and 6-11 may have been due to the abundant abscission of leaves from these trees. This assumption is supported by the data given in table 4 showing the number and dry weight of leaves in these series. Table 10 indicates that sodium and chlorin are not absorbed as molecular sodium chlorid but rather as Na-ions and Cl-ions.<sup>9</sup> The data for trees 13 and 16 indicate that the calcium has not prevented the entrance of Na or Cl-ions into the plant. Increasing the content of sodium chlorid in the culture solution increased the total absorption of both Na and Cl-ions (cf. table 10). Table 10 shows that a tree (tree 1) in Hoagland's solution which contains only a small concentration of sodium chlorid, may, because of its greater growth, actually

contain a larger amount of sodium in the entire tree than a tree (trees 13 and 16) growing for the same period of time in a culture solution relatively high in sodium.

Increasing the concentration of chlorin in the medium increases the total amount of chlorin in the tree, as well as the per cent present in the ash (except rootlets). This is well illustrated by the total amounts of chlorin in the trees (table 10). Trees 1, 2, 85, and 42-46 each received solutions containing 10 p.p.m. of chlorin, and their analysis (table 10) shows that they contained less total chlorin than trees 6-11 which received a solution containing 617 p.p.m. chlorin. Trees 6-11 likewise contained less total chlorin than trees 13 and 16 which received a solution containing 898 p.p.m. chlorin. The percentage of Na and of Cl in the ash of trees which received varying amounts of these ions is a matter of great interest in view of the physiological effect of these ions upon the trees.

TABLE 11  
PERCENTAGE OF SODIUM AND CHLORIN IN ASH OF TREES RECEIVING VARIOUS  
CULTURE SOLUTIONS

Culture solution contained	Trees 1 and 2	Trees 6-11	Trees 13 and 16	Trees 1 and 2	Trees 6-11	Trees 13 and 16
	7 p.p.m. Na	400 p.p.m. Na	400 p.p.m. Na	7 p.p.m. Na	400 p.p.m. Na	400 p.p.m. Na
	159 p.p.m. Ca	0 p.p.m. Ca	159 p.p.m. Ca	159 p.p.m. Ca	0 p.p.m. Ca	159 p.p.m. Ca
	10 p.p.m. Cl	617 p.p.m. Cl	898 p.p.m. Cl	10 p.p.m. Cl	617 p.p.m. Cl	898 p.p.m. Cl
Percentage found in ash						
	Na	Na	Na	Cl	Cl	Cl
Leaves.....	1.17	0.60	1.61	0.29	1.71	4.73
Shoots.....	2.56	1.43	1.14	0.13	5.55	5.64
Trunk.....	6.74	8.38	2.82	0.02	2.15	2.46
Root.....	3.74	13.90	7.73	0.13	2.84	3.34
Rootlets.....	0.92	9.75	5.30	1.01	13.21	8.54

The data in table 11 are assembled from tables 5 to 9 and from a previous paper<sup>2</sup> for convenience in making comparisons. Trees 1 and 2 received Hoagland's solution. The distribution of Na in trees 1 and 2 is by no means uniform. The trunk and root were richer in Na than any other portion of the tree. There seems to be a gradient extending from the trunk both upward and downward. Trees 6-11

show a somewhat similar distribution of Na. Here the maximum percentage was found in the root with a gradient extending toward the leaves. The large value for the rootlets may be somewhat misleading unless we mention that many of them were dead and might have held mechanically considerable amounts of the culture solution. Trees 13 and 16 show somewhat the same distribution of Na as trees 6-11. The percentage for the trunk and root combined was greater than for the rest of the tree. A very small percentage of Na was found in the leaves and shoots.

All parts of trees 1 and 2 contained a low percentage of Cl, the differences between the various portions of the tree being too small to warrant emphasis. There is no well marked gradient in any of the three series of trees. In contrast to the distribution of Na, we find the trunks and roots to be generally poorer in Cl than other parts of the trees. The leaves of trees 6-11 appear to be relatively low in Cl, which may be due to the immaturity of the leaves following the repeated abscissions already mentioned. The high value of Cl in the rootlets of these trees may be explained in the same manner as has already been done in the case of Na. The three series show a marked tendency toward higher percentages of Cl in corresponding parts of the trees as the concentration of this ion was increased in the culture solution.

The percentage of Ca in various parts of the tree is a matter of interest because it has been shown that successful growth was possible when  $\text{CaCl}_2$  was added to the culture solution containing 1000 p.p.m. NaCl. The data presented in table 12 show, first, that there is a fairly large amount of Ca in the ash of all parts of the trees receiving Hoagland's solution (trees 1 and 2); second, that when trees were grown in a medium to which no Ca was added (trees 42-46, 6-11), their ash contained smaller percentages of Ca; third, that trees 13 and 16, grown in a medium receiving 1000 p.p.m. NaCl plus the same amount of Ca as contained in Hoagland's solution, did not contain so high a percentage of Ca in their ash as those grown in unmodified Hoagland's solution.

The figures further show that the trunk and root in each case contained a higher percentage of Ca than the remaining portions of the trees. In the case of trees to which no Ca was supplied, it appears that the Ca of the trunk and root was relatively immobile. There-

fore, calcium in the ash of the shoots of trees receiving calcium-free culture solution, was extremely low. Rippel<sup>10</sup> has also shown that growing twigs are unable to resorb calcium and suffer more intensively from a lack of this element.

The lower percentage of Ca in trees 13 and 16 in comparison with trees 1 and 2 is a matter worthy of remark, since they were supplied with equal concentrations of Ca. It seems plausible to assume that this difference is perhaps due to the greater concentration of certain other ions in the culture solution with the consequent greater absorption of these ions.

TABLE 12

DISTRIBUTION OF CALCIUM

(Percentage of the ash)

TREES

Culture solution contained	1 and 2	42-46	6-11	13 and 16
	159 p.p.m. Ca	0 p.p.m. Ca	0 p.p.m. Ca.	159 p.p.m. Ca.
	Percentage of Ca found in the ash			
Leaves.....	18.71	1.28	.91	8.77
Shoots.....	21.98	2.98	3.48	16.07
Trunk.....	24.34	16.50	18.70	20.65
Root.....	23.42	13.49	14.60	17.94
Rootlets.....	18.88	4.55	3.56	16.64

During the winter of 1920-21 many leaves fell in each of the three series, the new leaves produced in series 12-17 being able to attain full size or maturity, while those leaves that were produced in series 6-11 and 42-46 were scarcely able to enlarge at all, most of them falling soon after becoming 1-3 cm. in length. The relatively young age of some of the leaves, together with the high potassium, sodium, and chlorin content of the culture solution, may all have played a part in causing the leaf ash of trees 13 and 16 to contain so relatively low a percentage of calcium (Ca). It is also possible that it was the low calcium percentage of the leaves of trees 13 and 16 rather than high percentages of chlorin or other ions that caused them to fall. It is much to be regretted that we know very little in regard to effects of ions such as Na and Cl once they have entered the sap of the tree.



It is significant that the leaves were somewhat mottled in several trees in each of three series (6-11, 42-46, 18-23) in which calcium was omitted from the culture solutions (pl. 6, figs. 1 and 2). The subject of mottling in citrus leaves is of such profound importance and the experimentally produced cases thus far so few in number, that it is unscientific to make any definite statement as yet regarding the cause of mottle-leaf. Schertz<sup>11</sup> found that under controlled conditions the mottling of *Coleus* cuttings is due to a deficiency of nitrate in the medium. As a result of certain experiments, Crocker<sup>12</sup> is of the opinion that a nitrate deficiency can scarcely be considered as a possible contributing cause of mottle-leaf in citrus.

The effect of lime, when applied to soil, upon the mottling of citrus trees, has been reported as being uncertain. Kelly and Cummins<sup>1</sup> after analyzing mottled and normal citrus leaves obtained in the field have shown that there is a deficiency of calcium in the ash of mottled leaves when compared with normal leaves. Even though calcium may be added to soils, it is unsafe to conclude that the rootlets have a supply adequate to their needs, since our knowledge of replacement phenomena in alkaline soils is still incomplete, as is also our knowledge regarding the chemical nature of the soil solution immediately in contact with the rootlets.

The results of these preliminary experiments with citrus trees grown in sand cultures have made it desirable to study various calcium relations of citrus under controlled conditions. Preliminary observations of cultures now growing in sand indicate that studies upon calcium deficiency may eventually throw some light upon the process of mottling in citrus. Experiments carried on at Wisconsin<sup>14, 15, 16</sup> have shown that one of the chief functions of calcium within the plant is to neutralize organic acids. This has an interesting application here, since mottled leaves which are deficient in calcium contain more unneutralized acid than normal citrus leaves. Kelley and Cummins<sup>1</sup> showed that 10 c.c. of normal leaf sap required 3 c.c. of  $\frac{N}{10}$  alkali for neutralization, while 10 c.c. of mottle-leaf sap required 7.05 c.c. of  $\frac{N}{10}$  alkali.

From the analytical data here presented (tables 5 to 9), it is seen that the  $\frac{Ca}{N}$  ratio for the dry matter of the different parts of trees

13 and 16 ranged from .5 to 1.6, while in the trees receiving calcium-free culture solution the ratios for the leaves, shoots, and rootlets were less than 0.2, and those for the trunk and root were approximately the same as for the corresponding parts of trees 13 and 16. It is interesting to note that the trunk and primary root were the last parts of the trees to die, and it is in the trunk and root that the high  $\frac{\text{Ca}}{\text{N}}$  ratio is maintained to the last.

The distribution of magnesium in trees which received no calcium is shown in table 13. With one exception (the shoots), the percentage of Mg in the ash of trees grown without Ca was greater than in corre-

TABLE 13  
DISTRIBUTION OF MAGNESIUM  
(Percentage of the ash)

Culture solution contained	Trees 42-46	Trees 6-11	Trees 13 and 16
	0 p.p.m. Ca 54 p.p.m. Mg	0 p.p.m. Ca 54 p.p.m. Mg	159 p.p.m. Ca 54 p.p.m. Mg
	Percentage of Mg found in the ash		
Leaves.....	1.16	1.57	1.15
Shoots.....	1.79	1.93	2.36
Trunk.....	2.81	2.46	1.60
Root.....	3.34	2.80	1.56
Rootlets.....	5.95	3.25	2.10

TABLE 14  
DISTRIBUTION OF POTASSIUM  
(Percentage of the ash)

Culture solution contained	Trees 1 and 2	Trees 42-46	Trees 6-11	Trees 13 and 16
	159 p.p.m. Ca 185 p.p.m. K	0 p.p.m. Ca 496 p.p.m. K	0 p.p.m. Ca 496 p.p.m. K	159 p.p.m. Ca 496 p.p.m. K
	Percentage of K found			
Leaves.....	24.73	50.04	50.07	38.25
Shoots.....	16.04	44.86	43.48	27.23
Trunk.....	11.47	26.65	14.05	19.92
Root.....	11.15	24.11	9.66	14.55
Rootlets.....	18.57	36.71	27.27	19.36

sponding parts of trees grown with Ca, although each series received the same concentration of Mg. Pfeiffer, Rippell, and Pfothenauer<sup>17</sup> have reported that there may be a partial substitution of magnesium for calcium, although they consider calcium to be of greater importance in plant growth.

The results given in table 13 indicate a slightly greater absorption of Mg in the absence of Ca, but it is doubtful whether we can assume that this additional Mg performs any of the functions of Ca in the tree. In the absence of calcium it is logical to expect that the trees might absorb somewhat larger amounts of other divalent cations such as Mg.

The percentage of potassium (K) in the ash of the various parts of the trees is extremely high (table 14). Kelley and Cummins<sup>1</sup> found that the ash of one-week-old Valencia orange leaves contained approximately 20 per cent of potassium (K), 13 per cent at six weeks of age, and amounts decreasing thereafter with increasing age. In the present experiments the analytical error in the determination of potassium may be considerable on account of the large amounts present and the large factors used in the calculations. If this should prove to be so, the percentage of potassium (K) would still be very high, especially in the leaves and shoots. It should be stated that very young growth was always omitted from samples used for analysis because of its high percentages of certain ash constituents and their consequent effect upon the analytical results of the mature leaves. When the trees had access to calcium, the percentage of potassium (K) in the ash of all portions, except trunk and root, was somewhat lower than when calcium was absent.

## SUMMARY

1. The physiological and chemical aspects of the effects of sodium chlorid and calcium salts upon absorption by orange trees are here reported. The trees were grown sixteen months in sand cultures under conditions which permitted careful control of factors affecting growth. The percentages of nitrogen and ash in the dry matter are greatest in the leaves, diminish as we pass to the trunk and root, and then increase as we pass to the rootlets. The trunks and roots of the trees were richer in sodium and poorer in chlorine than any other

part of the tree. The trunks and roots were richer in calcium than other parts of the tree. In trees to which no calcium was supplied it appeared that the calcium of the trunk and root was relatively immobile. The percentage of potassium shows a gradient from the leaves to the roots in each series.

2. The culture solutions, which were distinctly acid in reaction when freshly prepared, became more nearly neutral after being in contact with tree roots in sand. The reaction values of the sap of mature leaves from different series were quite similar. Differences in the initial osmotic pressure of the culture solutions were not reflected in the concentration of the leaf sap.

3. The young orange trees, a few months after the application of the modified culture solutions, began to show physiological disturbances, which became increasingly severe as time went on. The experiments were terminated before any of the trees died.

4. The lack of calcium resulted in severe losses of leaves from the trees. Shortly before their abscission the leaves were covered with small brown spots. Following the abscission of one lot of leaves, the new leaves frequently were chlorotic. The repeated stoppage and starting of new growth led to the condition known as "multiple buds." A few of the older leaves showed a certain type of mottling quite similar to, but not identical with that occurring in the field. The young shoot tips and their leaves lost their turgor and died. Ultimately the trunk began to die, beginning at the top. The root system of such trees was poorly developed, and the younger roots became gelatinous. In the presence of suitable amounts of calcium chlorid the trees made vigorous growth even though they also received considerable amounts of sodium chlorid.

5. An increase in the concentration of chlorin in the culture solution was followed by an increase in the percentage of this constituent in corresponding parts of the trees. A tree receiving a culture solution containing a very small concentration of sodium chlorid may, because of its greater growth, actually contain a larger amount of sodium in the tree as a whole than one receiving a relatively higher concentration of sodium chlorid.

6. The percentage of calcium in the ash of the different parts of the trees which were grown in a full nutrient solution was fairly constant. When calcium was lacking from the culture solution, the

ash of leaves, shoots, and rootlets contained very small percentages of calcium in comparison with the ash of trunks and roots, a fact which may indicate a relatively low mobility of this constituent in these parts. The trees which received a culture solution containing 1000 p.p.m. NaCl plus the full amount of calcium, contained somewhat smaller percentages of calcium in the ash of the various parts than trees receiving a culture solution containing only a small amount of NaCl.

7. A slightly greater absorption of magnesium generally occurred when calcium was absent from the culture solution. The percentage of potassium in the ash of the various parts of the trees was extremely high. When the trees received calcium in their culture solution, the percentage of potassium in the ash of all portions except trunks and roots was somewhat lower than when calcium was absent.

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## PLATE 1

Fig. 1. Leaves typical of trees 42-46 and 6-11 (calcium omitted from culture solution). At the time of abscission, the leaves may be in any of these stages. (Scale of inches at bottom of picture.)

Fig. 2. Young leaves from tree 7 produced after the abscission of leaves like those shown in figure 1. These pale leaves showing large numbers of minute dead spots were typical of trees grown with an extreme calcium deficiency. The effect shown here was typical of series 6-11 and 42-46.



Fig. 1



Fig. 2







PLATE 2

Fig. 1. Young shoot tips dying from extreme calcium deficiency.

Fig. 2. Shoot tips nearly dead from extreme calcium deficiency. The condition known as "multiple buds" is shown by these shoots.

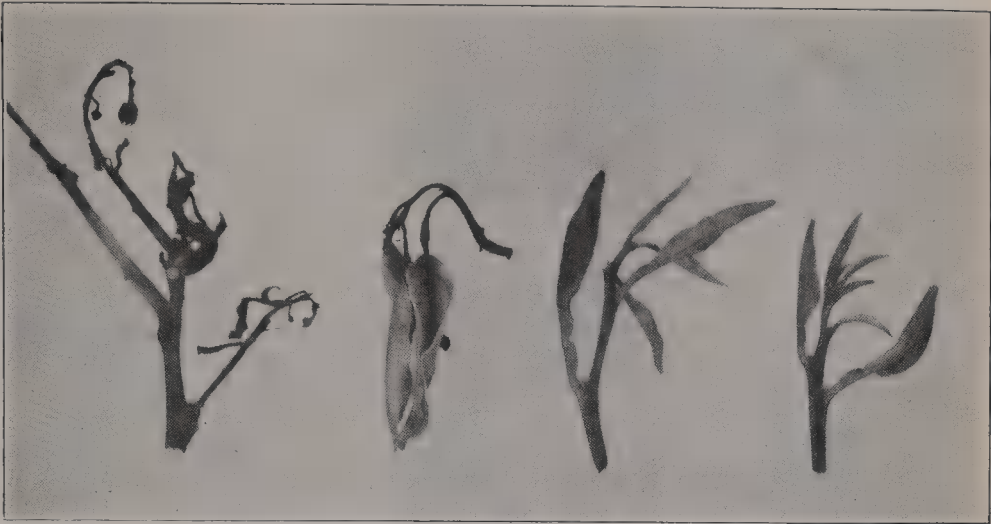


Fig. 1



Fig. 2





### PLATE 3

Fig. 1. The effect of calcium deficiency upon the type of leaf curling. Two specimens to the left taken from tree 42 (extreme calcium deficiency); specimen to the right shows normal method of leaf curl in Valencia orange leaves.

Fig. 2. Appearance of trees in series 42-46 which received Hoagland's solution in which potassium was substituted for calcium. May 21, 1920, to August 25, 1921.





Fig. 1



Fig. 2





#### PLATE 4

Fig. 1. Appearance of trees in series 6-11 which received Hoagland's solution in which potassium was substituted for calcium plus 1000 p.p.m. NaCl. May 21, 1920, to August 25, 1921.

Fig. 2. Appearance of trees in series 12-17 (tree 13 in the center). These trees received the same amount of potassium and sodium chlorid as those in series 6-11 (fig. 1), with the addition of 159 p.p.m. calcium in the form of calcium chlorid. May 21, 1920, to August 25, 1921.



Fig. 1



Fig. 2







## PLATE 5

Fig. 1. Root systems of trees 7 and 9 (typical of trees suffering from calcium deficiency) compared with that of tree 1 which received Hoagland's solution in sand culture.

Fig. 2. Root systems of trees shown in plate 4, figures 1 and 2. Root 9 grew in a culture which received Hoagland's solution in which potassium was substituted for calcium plus 1000 p.p.m. NaCl. This root was typical of those grown in series 42-46 which received the same solution as 9 minus the 1000 p.p.m. NaCl. Roots 13 and 16 grew in cultures receiving the same solution as 9 plus 159 p.p.m. calcium as calcium chlorid.



Fig. 1



Fig. 2







## PLATE 6

Fig. 1. Upper row of mottled leaves taken from experimental trees series 6-11, 18-23, 42-46 receiving calcium-free culture solution.

Lower row of mottled leaves taken from trees in plot C, Rubidoux tract, Citrus Experiment Station, Riverside, California. September 20, 1921.

Fig. 2. Upper row of four mottled leaves taken from trees in plot U, Rubidoux tract, Citrus Experiment Station, Riverside, California. September 20, 1921.

Lower row of four mottled leaves collected from experimental trees series 6-11, 18-23, 42-46 receiving calcium-free culture solution.



Fig. 1



Fig. 2



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Citrus blast and black pit, which were formerly considered to be due to two different species of bacteria, are now known to be simply different manifestations due to the same organism, *Pseudomonas citriputeale* C. O. Smith.<sup>1, 2</sup> Citrus blast is the name which was given to an effect of this bacterium on the leaves and twigs, and black pit the name given to a different effect upon the fruit.

The first of these effects to be reported was black pit, described by C. O. Smith in 1913<sup>2</sup> as a brown or black sunken spot occurring on lemon fruits grown in southern California. His attention had been first called to the disease in 1909. The causal organism was named by him *Pseudomonas citriputeale*.<sup>2</sup> Citrus blast was first described as a new disease of leaves and twigs by Coit, 1916<sup>3</sup> Hodgson reports having observed it as a new disease as early as 1912.<sup>4</sup> The organism was found on citrus blast lesions and named in 1917 *Bacterium citra-refaciens* by H. A. Lee.<sup>5</sup>

It is the purpose of this bulletin to report the results of investigations begun in 1919<sup>6</sup> to determine more definitely the nature of the disease, the conditions influencing its development, and the means to be employed for its prevention and treatment.

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\* Paper No. 102, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

## DISTRIBUTION

The disease was first thought to be confined principally to the citrus orchards of northern California, especially those of Butte County. It was afterwards found to be fairly well distributed throughout northern California, and a few cases were found in Tulare County. The phase of the disease on the fruit known as black pit, which was considered at first to be a different one, had been found by C. O. Smith in various localities in southern California, but the typical lesions on the twigs and leaves were not definitely observed in southern California until the past year.

It now appears evident that the organism responsible for these two manifestations is rather generally distributed throughout the sections in which citrus is grown, and that the manifestations on the leaves and twigs known as blast are especially dependent for their occurrence upon the right combination of weather conditions with suitable mechanical injuries. In the spring of 1922, one of the groves highest up on the foothills near Pasadena showed a considerable outbreak of blast upon the twigs and leaves, and of black pit upon the fruit. The rainfall in this orchard between January 1 and May 1 was about thirty inches, and this, together with the unusual number of cool, cloudy days, produced conditions which were quite similar to those which occur on the foothills near Oroville, where the citrus blast is usually severe. It therefore seems likely that both citrus blast and black pit may appear whenever suitable weather conditions occur.

## SYMPTOMS AND DEVELOPMENT

As stated above, this disease occurs in two forms, one on the leaves and twigs and the other on the fruit. Its activity is confined to a limited period, usually extending over six to eight weeks in the winter and spring. Oranges and grapefruit are probably the most susceptible to the injury upon the leaves and twigs, while lemons are the most susceptible so far as the fruit is concerned.

The most striking characteristic of the blast manifestation is an area shading from brown to black, which usually starts in a tear or break on the wing of the petiole (see pl. 1, fig. A) and extends rapidly in both directions to the base of the leaf blade and to the twig surrounding the base of the petiole. On January 22, 1921, at Oroville, out of thirty-eight lesions taken at random thirty had started on the wing of the petiole at some place between the leaf blade and twig; seven had started at the base of the leaf blade; and only one had originated on the twig near the base of the petiole. During cool, damp weather these areas enlarge rapidly, but when the dry and warmer weather comes on the progress of the lesions is checked. The following features are to be noted: there is a definite line between the dead and live tissue; new callus tissue forms under the affected areas; the surface of the affected bark becomes dry, and the initial black color changes to a reddish brown, forming scabs (pl. 1, figs. B and C). If the disease progresses very rapidly the leaves wither and die while still attached to the tree (pl. 2), but if its progress is slow or if it occurs in a somewhat warmer temperature, an abscission layer is formed and the leaves are shed. In the latter case, it seldom progresses farther than the petiole of the leaf, and few lesions will be formed on the branches. In very severe cases the disease may progress so rapidly as to girdle the twigs and cause many of them to wither and die; but more frequently the lesions are checked before girdling takes place. Microscopic examinations have shown that the bacteria are most abundant in the layers not far from the cambium. They appear to attack the parenchyma tissue principally, and do not kill the woody tissue except in extreme cases.

A corky brown scorching, or staining, of the surface layers of leaves often accompanies the blast. This sometimes spreads over a portion of the leaf blade beyond the edge of the lesion (pl. 3, figs. A and B). Its occurrence under the same conditions as those which produce blast has indicated that the citrus blast organism may have some relation to this manifestation. It differs from the ordinary form of blast in that only the very outermost layers of cells are affected, while the affection does not cause the death of the tissue beneath. Some experiments have shown that a reddish brown scorching similar to that observed can be produced by rubbing the under surface of the leaves with a blunt instrument and painting on a suspension of the



organism. The leaves which were rubbed without painting with this organism simply took on a grayish discoloration without any reddish brown color.

As black pit the disease manifests itself on the fruit, especially in the case of lemons, as sunken black spots (pl. 5). As these develop they change from a light brown to dark brown, and then to black. The inner white part of the peel is also affected. There is a collapse of the tissue, which finally becomes light brown tinging to reddish brown in color. The size of these spots is dependent upon the conditions of moisture and temperature under which they develop. On lemons they are mostly from one-fourth to one-half an inch in diameter, but many attain a size of three-fourths of an inch. If the spots occur close together, they may be smaller. These black pit spots may also occur on oranges and grapefruit (pl. 4), but are usually not as common nor as deep as upon the lemon fruits. As in the case of citrus blast, the black pit spots are usually dependent upon injuries.

## IDENTITY OF CITRUS BLAST AND BLACK PIT ORGANISMS

While work was being done with cultures of the citrus blast organism obtained from northern California in the spring of 1920, isolations of bacteria also were made from some black pit spots on lemons obtained from Alta Loma in southern California. The similarity in the appearance of the cultures from the two sources was noticed, and parallel inoculations were made on lemon fruits by puncturing them on one side with the organism from citrus blast lesions and on the opposite side with the organism obtained from black pit. Black pit spots, identical in their manner of development, were obtained in both cases. These results led to the suspicion that we were dealing with the same species of organism and not with two different species, as had formerly been supposed. Experiments of this kind were repeated several times with the same results (pl. 5). It was also found that typical blast lesions could be produced on short shoots of citrus kept in moist chambers in the laboratory. Parallel inoculations with these organisms from the two different sources showed them to have the same effect in producing blast lesions on these cuttings. Some of these experiments were repeated at Berkeley by A. F. Camp, both in



the laboratory on fruit and in the greenhouse on growing plants, with the same results. Later, at Oroville, the organisms from two different sources were again tested by a number of inoculations, both in moist chambers and in the orchard. The results here again indicated the identity of the organisms from the two sources. Since that time the organism has been isolated repeatedly from twig lesions and upon being used for inoculation has been found to produce black pit. Likewise, it has been isolated repeatedly from black pit lesions and caused to produce the blast lesion on twigs (pls. 5 and 6).

*Culture and Staining Tests.*—The organisms from the two sources (blast lesions of orange twigs and black pit lesions of lemon fruits) have been carefully compared in parallel cultures and in staining tests. Most of the tests reported both by C. O. Smith and H. A. Lee were repeated and others added. In all these tests the cultures from the two sources were alike in their reactions. No differences that could possibly separate them as species were detected. The organisms from the two sources were also compared on a few of the standard media with one of C. O. Smith's original cultures, which have been kept alive by transfers since 1913. The results were identical. We have found the characteristics of the organisms to agree with Smith's description of *Pseudomonas citriputeale*, with the exception of the number of flagella, which is usually few to many, instead of one. Lee found one to four, but there are usually many more than four.

The results of our tests differ from Lee's technical description of *Bacterium citrarefaciens* in at least two particulars, and from his general description in several others. We found, as did Smith, no coagulation in milk, and the green color mentioned by Lee as a prominent characteristic was absent in almost all media. Only rarely, and only on standard agar containing considerable glucose, was there even a slight suggestion of green, and then only with certain strains of the organism. It is possible that Lee was dealing with a strain of the organism with an exceptional tendency toward the production of a green pigment. In other respects our results differed from Lee's in that no growth was obtained either in Uschinsky solution or in the closed arm of fermentation tubes of maltose. Our results agreed with Lee's in that growth was obtained in the closed arm of tubes with dextrose and saccharose, and none in the closed arms with lactose. In most other respects our results were also in agreement with Lee's general description.

The organism from the two sources produced practically the same effect on the H-ion concentration of various culture media. In various sugar solutions (two per cent) in fermentation tubes with an initial pH of about 7.4, the comparative results are as shown in table 1.

TABLE 1

Source	pH in 34 days in			
	Saccharose	Dextrose	Maltose	Lactose
Blast.....	4.4	6.8	6.8	7.4
Black pit.....	4.5	6.8	6.8	7.6

*Inoculation Tests.*—Inoculations were made May 17, 1920 on lemon fruits, to compare the effects of bacteria isolated from citrus blast lesions on orange twigs from northern California with that of bacteria isolated from black pit lesions on lemon fruits from southern California. Four lemons (two light green and two yellow) were placed in each of seven temperature chambers after being inoculated. One side of each fruit was inoculated by puncturing with a steel needle in four different places in the case of the northern organism, and the other side inoculated in a similar way in the case of the southern. The effects produced by the organisms from the two different sources were quite similar, as shown in table 2.

TABLE 2

DIAMETER OF BLACK PIT SPOTS ON LEMON FRUITS THREE DAYS AFTER INOCULATION  
(Average in mm. of six to eight punctures on each of two fruits)

Source of cultures	Kind of fruit	14° C. 57° F.	17° C. 64.5° F.	20° C. 67° F.	24° C. 75.3° F.	27° C. 80.6° F.	30° C. 86° F.	33° C. 91.5° F.
Blast lesion, Oroville.....	Light green	7.0	5.4	6.2	3.7	3.2	1.0	0
	Ripe.....	5.2	7.2	7.9	4.9	6.0	1.6	0
Black pit lesion, Alta Loma.....	Light green	6.5	5.4	5.7	3.3	4.8	1.8	0
	Ripe.....	5.4	7.1	4.7	3.7	5.6	1.6	0

No lesions developed at 33° C. (91.5° F.). At 30° C. (86° F.) there appeared to be a slight temporary effect, but no development of lesions. At 27°, 24°, 20°, 17°, and 14° C. (80.6°, 75.3°, 64.5°, and 57° F.) black pit lesions developed. In three days the largest spots in diameter on green fruit were produced at 14° C. (57° F.), but those on the fruits kept at 17° and 20° C. (64.5° and 67° F.) were

nearly as large and more sunken. The largest spots developed on ripe fruit at temperatures of 17° and 20° C. (64.5° and 67° F.). A fairly cool temperature, such as 14 to 20° C. (57 to 67° F.) is most favorable for the development of lesions, but lesions may develop at constant temperatures as high as 27° C. (80.6° F.). In general, the organisms from the two sources acted alike in producing black pit.

A comparison of the size of colonies of the bacteria from blast and black pit lesions in different temperatures after three days is shown in table 3. The inoculations were made by bringing a one-millimeter loop from a bouillon culture in contact with the surface of solidified soluble-starch agar.

TABLE 3  
SIZE OF COLONIES (MM. IN DIAMETER) IN THREE DAYS, ON SOLUBLE STARCH AGAR  
PLUS ONE-HALF PER CENT GLUCOSE  
(Average of about six colonies in three petri dishes)

Source of culture	Temperature						
	13° C. 55.4° F.	17° C. 64.5° F.	20.5° C. 69° F.	24.5° C. 76° F.	27° C. 80.6° F.	30° C. 86° F.	34° C. 93.2° F.
Blast lesion, Oroville.....	4.3	6.0	8.0	4.8	3.4	3.0	0
Black-pit lesion, Alt. Loma.....	3.5	6.5	7.6	5.8	3.1	3.0	0

There appeared to be no significant difference between the cultures from the two different sources in their reaction to temperature. The largest colonies were formed at 20.5° C. (69° F.). No growth took place at 34° C. (93.2° F.).

EXPERIMENTS IN ISOLATING THE ORGANISM

The blast organism has been isolated not only from the leaf and stem lesions, but also from black pit spots on lemon, grapefruit, and orange fruits. It was also readily found in drippings both from infected and sound leaves during and following rains. The organism was isolated readily from active lesions, but not so easily from older lesions that had ceased enlarging.

Out of a considerable number of tests from healed-over lesions a year or more old, a few yielded organisms capable of producing black pit on lemon fruits. The results appear to point to a strong probability that the organism is carried over in some of these lesions

at least, even after the lesions are healed over and appear to be inactive. Lee reports having isolated the organisms from inactive lesions after the scabs had formed.

Certain cultural and inoculation tests, made to determine whether the organism was common in the soil and air in an infected orchard, failed to give any positive results. These tests were not extensive, but they indicated that the blast organism was not abundant in the particular samples tested, since the organism could be readily detected in other samples of soil, to which the blast organism had been purposely added.

### INOCULATIONS ON DIFFERENT SPECIES OF CITRUS AT BERKELEY

During September and October, 1920, a number of sets of inoculations comprising several leaves each were made at Berkeley on trees in a moist atmosphere in an outdoor inoculation chamber devised by Camp.<sup>7</sup> The surface of the leaves in this chamber was kept almost continually moist. Inoculations were made by puncturing with a needle and by hypodermic injection into leaves of sweet orange, sour orange, tangerine, and Eureka lemon. The temperatures in the chamber ranged from about 10 to 17° C. (50 to 62° F.). Positive results in producing blast lesions on leaves were obtained in the case of all these varieties, definite evidences of development starting usually in from about three to six days.

On the sweet orange the evidences of the development of the disease were noted in from three to six days. On growing and recently matured leaves they developed in from three to four days. One inoculation, which had begun within four days from a needle scratch, spread along the petiole and seven millimeters along the stem in twenty-one days. On older leaves a lesion which had started within six days had spread down the petiole and three millimeters along the stem in twenty-two days.

Recently matured sour orange leaves showed positive beginning of lesions in from three to four days, and more mature leaves in from seven to eight days. No striking difference in resistance to spread of lesions was noted between the sweet and sour orange.

Dancy tangerine leaves, recently matured, showed positive lesions in from three to six days in different experiments, and displayed the same characteristics as in the case of the sour or sweet orange leaves.

On rapidly growing Eureka lemon leaves, the evidences of development were seen in from three to eleven days. The spread of the lesions stopped sooner than on the orange leaves, indicating a greater degree of resistance to invasion of the organism. Leaves of all these varieties which were atomized but not punctured or injured failed to produce blast.

In April, 1921, hypodermic injections in the case of matured orange leaves started positive lesions in six days, but in the case of young leaves only slight or negative effects were produced.

#### DOES THE CITRUS BLAST ORGANISM ATTACK OTHER HOSTS?

While observations on citrus blast were being made in the Oroville section, lesions which resembled those on citrus trees were discovered on the leaves and stems of the interior live oak, *Quercus wislizenii*. These oaks were in the vicinity of orchards affected with citrus blast. Trees of the same species of oak growing far back in the foothills and mountains were examined, and were also found to contain these lesions. The lesions have not been found in great numbers on this oak, nor do they appear to damage the oak as severely as they do the citrus. Similar lesions were also found on the same species of oaks growing on the foothills in Tulare County. A number of isolation tests were made, but no cultures of the blast organism were obtained from these naturally occurring lesions. Active, fresh lesions on the oak have not been tested.

That lesions of this nature, however, may be produced on oaks by the citrus blast organism has been shown by the following experiments.

Small oak trees, *Quercus wislizenii*, were transferred to the lath-house of the Citrus Experiment Station and the following year inoculations with the citrus blast organism were made in punctures on the very new growth. Petioles of young leaves and growing stems of three plants were punctured with a hypodermic needle containing a suspension of the organism, and the plants were covered with bell jars. Lesions were produced similar to those which had been observed



as occurring on this species in the field. The organism was again isolated from these lesions, after they had developed for about eight days. Twigs of this oak, growing rapidly when cut, were also inoculated in moist chambers held at different temperatures. The best development of lesions was at 22.5° C. (73° F.) and occurred in five days. Definite lesions developed at 13° C. (55.4° F.) in nine days.

Lesions had previously been produced on the coast live oak, *Quercus agrifolia*, at Berkeley. Two leaves of this oak were punctured on April 5, 1920. Positive lesions had extended twelve millimeters along midrib and petiole and ten millimeters along the stem in seven days. The organism was then isolated from this lesion and produced black pit on lemon fruits.

In June, 1920, bits of tissue from lesions found on oaks, *Quercus wislizenii*, at Oroville were inserted in the rind of lemon fruits with the result that typical black pit lesions were formed.

These experiments and observations afford strong evidence that certain species of oaks common in California are attacked by the citrus blast organism. Lesions have been produced on the oaks by inoculation with the organism from the citrus, and cultures of the same organism have been recovered from these oak lesions. It remains, however, to isolate the organism from the naturally occurring lesions on the oaks, and to produce with it the blast upon the citrus. Although the later part of the proof has not as yet been obtained, the originators of the theory believe it is only a question of getting the oak lesions at the time of their most active development.

From March 23 to April 2, 1921, the following attempts were made at Berkeley to produce lesions on deciduous fruit trees with cultures of the blast bacterium.

(1) Cherry: Flowers and leaves atomized with suspension of the organism, terminal buds and leaves injected with hypodermic needle with suspension, young leaves and shoots punctured.

(2) Apricot: Young leaves atomized, buds and growing shoots injected with hypodermic needle.

(3) Pear: Young growing leaves atomized, buds injected.

(4) Almond: Young growing leaves atomized, buds and growing shoots injected.

All these tests failed. Positive lesions were, however, produced on mature sweet orange leaves under the same conditions.

RELATION OF CITRUS BLAST TO ENVIRONMENTAL  
CONDITIONS

RELATION TO MOISTURE

Observations have indicated that the severity of citrus blast injury was dependent to a considerable extent upon the moisture conditions during the early part of each year, usually from January to March. The disease was quite severe during some years and mild or of small importance in other years. It also seemed evident from observation that when the leaves were covered almost continuously for several days with a film of water during cool rains, the disease developed rapidly, while its development was slower when the weather was such as to allow the foliage to become dry. In accordance with Hodgson's observations from 1912 to 1918, and ours of more recent date, the past eleven years have been divided into two groups, one in which the blast was said to be severe and the other in which it was considered at Oroville to be mild. The following table shows the rainfall, number of days of rain and of totally cloudy days for the period between January 1 and March 31 for each of these years.

TABLE 4  
RAINFALL, NUMBER OF RAINY AND CLOUDY DAYS FOR JANUARY, FEBRUARY AND  
MARCH, AT OROVILLE, IN RELATION TO CITRUS BLAST

Citrus blast severe (years)	Rainfall	Days of rain	Cloudy days
1915.....	23.52	36	60
1916.....	20.28	44	55
1919.....	14.28	37	51
1921.....	11.15	30	23
1922.....	12.77	37	35
Citrus blast mild (years)			
1912.....	6.26	23	48
1913.....	9.16	20	27
1914.....	15.80	24	39
1917.....	10.01	19	27
1918.....	12.93	26	49
1920.....	7.26	23	38

TABLE 5

RAINFALL, NUMBER OF RAINY AND CLOUDY DAYS FOR JANUARY, FEBRUARY AND MARCH, AT RIVERSIDE, WHERE LITTLE OR NO CITRUS BLAST OCCURRED, DURING THE SAME PERIOD AS SHOWN IN TABLE 4

Years	Rainfall	Days of rain	Cloudy days
1915.....	8.6	21	18
1916.....	12.47	28	10
1919.....	4.60	18	13
1921.....	5.03	16	14
1922.....	8.58	22	14
1912.....	.75	15	12
1913.....	4.19	5	4
1914.....	10.01	20	20
1917.....	4.25	17	13
1918.....	8.32	18	12
1920.....	7.74	23	21

It is seen that the records for the number of rainy days in the severe group of years at Oroville are higher than those in the mild group of years. This feature of the weather is probably the most important in its influence on citrus blast. The number of totally cloudy days in general was higher in the severe years than in the mild years and this is also true of the rainfall. In 1921, one of the severe years, when the rainfall was comparatively light while the number of rainy days was fairly large, the blast appeared early because of heavy December rains which are not included in this record. In 1914, one of the mild years, the rainfall for the period was fairly heavy, but the number of rainy days remained few and was small in February and March. A study of the weather data for the same period at Riverside shows that in only one year, 1916, of the eleven recorded, did the number of rainy days approach even the lowest figure for the severe years at Oroville, and in most years it was below the average for the mild years at Oroville. The highest number of cloudy days at Riverside for the period covering the eleven years, was considerably below the lowest at Oroville. This is probably one of the main factors in the situation, at least it may account for the slight occurrence of citrus blast in sections like Riverside.

That the number of rainy days may be an important factor is further borne out by the fact that this past year citrus blast occurred to a considerable degree in an orchard on the foothills below Mt.

Wilson near Pasadena, where the rainfall from January to May was about thirty inches, accompanied by much fog, severe winds, and cool weather resembling that of normal years at Oroville. The strong winds producing the necessary injuries near Pasadena were from the northeast, and it was also on the northeast side that the majority of the citrus blast lesions were found.

RELATION TO INJURIES

After a careful study of the development of the lesions and of inoculation tests, it has been determined that infection leading to development of blast or black pit usually takes place in slight rifts, tears, scratches, thorn pricks, scars, rubbings, or other injuries and does so most readily if these injuries are fresh and kept moist by rains or fogs. The data in tables 6 and 7 show the percentage of

TABLE 6

PERCENTAGE OF INJURED LEAVES AT OROVILLE ON TEN SIX-YEAR-OLD TREES WITH LONG, VIGOROUS SHOOTS

Date	Number of twigs	Total number of leaves counted	Number of leaves injured	Percentage injured
<i>February 8, 1921</i>				
South half.....	47	751	468	62
North half.....	52	764	420	55
<i>February 15, 1921</i>				
South half.....	44	654	390	60
North half.....	39	485	242	50

TABLE 7

Date	Leaves injured			Leaves not apparently injured		
	Total number	Number with blast	Percentage of blast	Total number	Number with blast	Percentage of blast
<i>February 8, 1921</i>						
South half.....	468	317	67	283	0	0
North half.....	420	165	37	344	0	0
<i>February 16, 1921</i>						
South half.....	390	266	68	265	2	0.8
North half.....	242	124	50	248	5	2.0

injured leaves on similar twigs taken to represent the north and south half of the tree and also the corresponding percentage of blast.

The percentage of leaves injured, as shown in table 5, was somewhat less on the north than on the south side of the trees. These trees were of an open type of growth. More compact trees would probably have shown very striking differences in the two sides. The blast lesions which had developed from injuries were also considerably less abundant on the north side, away from the prevailing winds, than on the south side. Calculation shows in the one case that 41.5 per cent of the leaves on the south were diseased, as compared with 20.3 per cent on the north side; and in the other set, 40.8 per cent of leaves on the south side as compared with 25 per cent on the north side.

An analysis of the kind of injuries on the leaves and their relation to citrus blast was also made on February 15, 1921. The data are shown in table 7.

TABLE 8  
KIND OF INJURIES AS RELATED TO CITRUS BLAST AT OROVILLE, FEBRUARY 15, 1921

Kind of Injury	Leaves with blast			Leaves without blast		
	South	North	Total	South	North	Total
Breaks: mostly a rift in one wing of the petiole.....	221	111	332	26	26	52
Punctured or torn places, mostly in the leaf blade.....	15	6	21	2	3	5
Rubbed places.....	6	2	8	3	0	3
Injuries by Fuller's rose beetle.....	24	5	29	92	84	176
No apparent injuries.....	2	5	7	262	243	505
Grand total.....		397			741	

It is here seen that the main portion of the citrus blast lesions developed from a certain type of injury in the wing of the petiole such as is shown in plate 2, figure A. The disease may also develop in other punctured or torn places in the leaf blade, in rubbed places, and in injuries made previously by Fuller's rose beetle. In this last case, however, the proportion of lesions to the whole number of such injuries was rather small, probably because of the fact that they were made before the active period of the blast began. A few lesions were found where no apparent injury could be detected with a hand



lens, although it was not ascertained with absolute certainty that none of the cells had not been previously injured.

In contrast to these records, which were taken from trees exposed to the wind, a total of 712 leaves was counted on similar growth on the south side of trees protected from south wind by large olive trees. Of these, only thirty-four, or about 8 per cent, were injured. Lesions had developed in thirty-one instances, or 23 per cent, of these injuries.

RELATION TO TEMPERATURE

Temperature, especially within certain limits, also has an important influence on development of citrus blast, as shown in tables 2 and 3. In an orchard under natural conditions it is especially difficult to estimate the influence of temperature apart from humidity, since the latter usually varies with the former. The temperature, of course, influences the host as well as the parasite, which makes an analysis of the temperature influence difficult.

In an orchard in the Wyandotte section where blast was developed actively in 1921, temperature records showed the following average daily maximum and minimum temperatures for January, February, and twenty-four days in March.

TABLE 9  
AVERAGE TEMPERATURES IN ORCHARD NEAR OROVILLE, WHILE CITRUS BLAST  
WAS ACTIVE

	Maximum (F.)	Minimum (F.)
January.....	50°	37°
February.....	64°	45°
March 1-24.....	77°	52°

The humidity record, which was kept during February, showed an average maximum of 96 and an average minimum of 53.

It has been observed in Butte County that in the spring, soon after the weather becomes warmer and dryer the active development of lesions no longer takes place. It is probable that a change in the host may be an important factor in this instance, as it is in so many plant diseases; but temperature is also thought to be of importance. In

addition to the influence of the temperature during development of the disease, that of previous chilling of the host appears to be a factor in some cases, although it is not a necessary condition for disease development.

An attempt has been made to obtain some light on the temperature relations by investigating the development of the organism and the lesions in artificial inoculations in constant-temperature chambers. Some experiments in the transfer of inoculated tissue from one temperature to another have been also carried on. Inoculations of cut twigs from recent growth of sour and sweet orange were made on February 24, 1920, and put into moist chambers in a supposedly saturated atmosphere. Needle punctures containing blast bacteria were made on these twigs near the base of petioles, and check punctures without inoculum were made on other twigs. In two days there was definite indication of development of the disease at 13, 16.5, 19, 22.5, and 26° C. (55.4, 62, 66.5, 73, and 78.8° F.). In four days the largest lesions, which were two to four millimeters in their longest diameters, were produced, the temperature being 19° C. (66.5° F.). Slight discoloration at punctures and gum exudation was noted at 29 and 33° (84.2 and 91.5° F.). The checks at this temperature showed no gum. No gum or other indication of disease was noted at 40° C. (104° F.). At this temperature the leaves turned brown in four days, as if they had been cooked. The sour orange shoot appeared to resist the heat longer than the sweet orange shoot. The moist chambers containing the twigs were then taken out of their respective temperature chambers and placed together in the laboratory at about 16 to 20° C. (60.8 to 68° F.). Within six days the greatest lesions appeared to have developed on twigs which had previously been kept at 13° C. (55.4° F.). On these the lesions were large and definite, being five to seven millimeters in their longest diameter. The sour orange tissue appeared to be slightly more resistant to the development of lesions than the sweet orange.

Another set of somewhat older cuttings of sweet orange were inoculated on March 29, 1920, and developed their largest lesions in five days at 22.5° C. (73° F.); while those kept at 13 and 19° C. (55.4 and 66.5° F.) were also well started. Gum exudation took place at 26° C. (78.8° F.). In nine days the lesions at 13° C. (55.4° F.) were very marked. Cuttings of recently grown twigs of

*Quercus wislizenii* inoculated at the same time also developed definite lesions at 22.5° C. (73° F.) in five days and at 13° C. (55.4° F.) in nine days.

On April 27, 1922, inoculations were made on yellow lemon fruits by transferring a two-millimeter loop full of bouillon culture and puncturing the surface of the rind through this drop. The fruits were then placed in moist chambers at different temperatures. The average sizes of spots developing in three and in five days, respectively, are shown in table 10.

TABLE 10  
SIZE OF BLACK PIT LESIONS DEVELOPING IN FIVE DAYS AT DIFFERENT  
TEMPERATURES  
(Average of about twenty-four punctures on four yellow lemon fruits  
in each temperature)

	Temperature						
	8.5 47	12.5 54.5	17 62.6	20.5 69	25 77	28.5 83.4	33.5° C. 92.5° F.
Size of lesions in mm. after 3 days.....	2.0	3.5	6.6	6.6	6.3	3.3	3.1
Size of lesions in mm. after 5 days.....	2.33	5.1	8.0	6.6	6.3	3.3	3.1

The spots averaged about equal as to diameter on the third day, the temperature being 17, 20.5, and 25° C. (62.6, 69, and 77° F.). They were more sunken at the highest temperature. In five days the spots developing at 17° C. (62.6° F.) were decidedly larger than at any other temperature. Those developing at 20.5° C. (69° F.) or above did not increase after the third day. Those at 33.5° C. (92.5° F.) were not typical, deep-seated spots, and probably developed rapidly previous to the death of the organism. Those at 28.5° C. (83.4° F.) did not enlarge after the second day.

On May 10, 1922, an experiment with recently grown twigs from navel orange trees was started. These were inoculated by cutting through the wing of the petiole with a scalpel carrying the organism and by puncturing, and were kept in a saturated atmosphere, as in previous experiments. In five and seven days the most pronounced lesions were found to have developed at 17 and 20.5° C. (62.6 and 69° F.). Small lesions with gumming and abundant callus were pro-

duced at 28.5 and 33.5° C. (83.4 and 92.5° F.). Lesions were quite definite at 12.5° C. (54.5° F.). Slight but definite development also occurred within this time at 7.5° C. (47° F.). At the end of seven days some of the twigs which had been at 7.5° C. (47° F.) were placed in a temperature of 20.5° C. (69° F.), and some of those at 20.5° C. in a temperature of 7.5° C. In six days more those transferred from 7.5° C. to 20.5° C. had increased rapidly, forming large individual lesions about equal in size to those which had remained for the entire time at 12.5° C. Those transferred from 20.5 C. to 7.5° C. developed very little during these six days, while the lesions developed at 12.5° C. were the most distinct and measured about five by two millimeters. These experiments indicate that temperatures of about 20° C. (68° F.) or below are most conducive to the development of citrus blast lesions, and that lesions may develop well at a constant temperature of 12.5° C., while they develop slowly at temperatures as low as 8.5° C.

There is also a suggestion that chilling followed by higher temperatures may render the tissues more susceptible to the invasion of the organism. This is in keeping with the observation that citrus blast is severe after periods of low temperatures not sufficient to freeze the tissue. The development of the citrus blast lesions prevalent in southern California this year (1922) may have been promoted by the low temperatures which prevailed from January 18th to 22nd, and were followed by a large number of rainy days and moderately cool weather and winds in March and April.

Inoculations made by injecting bacteria with a hypodermic needle into the leaves of sour orange one-year-old seedlings, growing in pots, were made at different temperatures on May 9, 1922. The trees had just begun new growth, but the injections were made in the older leaves. One plant with three leaves injected was placed at each of the following temperatures and given artificial light for nine hours each day: 8, 12.5, 17, 21, 24, 28.5, and 33° C. (47, 54.5, 62.6, 69, 77, 83.4, and 92.5° F.). Checks were injected with distilled water and kept at 8 and 21° C., but produced no spots. Dead spots were produced by the inoculations at all these temperatures. There was no enlargement after the second day in the case of leaves at 33° C. (91.5° F.), and none after the third day in the case of those at 28, 24, or 21° C. (83.4, 75.2, or 70° F.). The lesions on those at 17, 12.5,



and 8° C. (62.6, 54.5, and 46.6° F.) continued to increase in size up to the eighth day, when the plants were taken out and all placed at a temperature of 18 to 20° C. (64.5 to 68° F.). In the next six days the lesions on the one which had been at 12.5° C. (54.5° F.) increased rapidly and caused more injury than any of the others. Those on the one which had been at 17 and 8° C. (62.6 and 46.6° F.) also increased somewhat. Those on the one which had been at 21° C. (70° F.) or above failed to increase after removal from the chambers, and the lesions appeared the same size as they were at the end of the third day. The spots on the leaves at the higher temperatures varied from dark to reddish brown, and those on the leaves at the lower temperatures from dark brown to black in color.

Culture tests on glucose potato agar, made by stabbing the surface of the solidified medium in petri dishes with the end of a needle, showed the largest colonies at 24° C. (75.2° F.) in one, two, and three days. In five days the colonies were almost the same size as they were at 17, 20, 27, and 29° C. (62.5, 68, 80.5, and 84.2° F.), but very small and they were probably dead at 33° C. (91.5° F.). A fair growth took place at 10 and 14° C. (50 and 57° F.). The cultures from blast lesions in the north reacted in a similar manner to the culture from black pit lesions of the south.

## CONTROL

### EXPERIMENTS ON PREVENTION

Spraying experiments have been carried on during three consecutive years, and the results each year have indicated that Bordeaux mixture, if applied as early as the first of November, will prevent a considerable part of the injury from this disease. It is of small advantage if applied some weeks later. None of the other mixtures tried, with the exception of mercuric cyanid (one part to 500 parts of water), were nearly as effective.

On October 28, 1919, a block of sixteen navel orange trees at Oroville were sprayed with Bordeaux mixture (5-5-50) and another block of twelve trees with mercuric cyanid (1-500). These trees were five years of age, and were surrounded by similar trees of the same age, but unsprayed. They contained a number of old scabs



from the lesions of the previous year. The new activity for that season at Oroville, however, was not noted until the middle of January, 1920. The results are partially indicated in table 11.

TABLE 11  
NUMBER OF AFFECTED TWIGS PER TREE. SPRAYED OCTOBER 28, 1919

No. of trees	Treatment	1920		
		January 24	March 25	May 25
16	Bordeaux 5-5-50	1.5	5.4	14.8
12	Mercuric cyanid 1-500	1.7	7.0	12.7
19	Check	9.8	22.7	33.6*

\* In this later count fifty-five trees were included as checks.

These figures give only a part of the results, since the twigs on the sprayed trees which did become affected were much less severely affected than twigs on the unsprayed trees. The sprayed trees stood out in striking contrast to the unsprayed trees, as being only slightly damaged. Almost no twigs on the sprayed trees were girdled and killed, while the unsprayed trees showed many twigs that had been killed back.

A very slight burning of leaves was noticed on the trees sprayed with mercuric cyanid, but none on the trees sprayed with Bordeaux. Otherwise the general appearance of the trees was similar in the case of the two sprays. On eight trees of the Bordeaux plot and six of the mercuric cyanid plot, most of the dead twigs and old lesions of the previous year were dissected out just following the spraying. No appreciable difference could be detected as a result of this treatment. These trees, however, were surrounded by trees full of old lesions.

Another block of trees were sprayed with a three per cent Ortho lime-sulfur solution on October 29, 1919. In this block no difference could be detected between the sprayed and unsprayed trees. Other trees were sprayed with Bordeaux at later dates, some in January, 1920, and some in February. In the case of these later sprayings little difference could be detected between the sprayed trees and the unsprayed ones adjacent to them.

On December 17, 1919, experiments with soda Bordeaux (2 lbs. sal-soda, 2 lbs. copper sulfate, to 50 gallons of water) and Bordeaux were made in another orchard of navel orange trees about ten years of age. Records, which were taken on January 24 only, showed the following results (table 12) :

TABLE 12  
NUMBER OF AFFECTED TWIGS PER TREE. SPRAYED DECEMBER 17, 1919

No. of trees	Treatment	Jan. 24, 1920 (p. 69-70)
8	Bordeaux.....	10.5
9	Soda Bordeaux.....	10.7
10	Check.....	14.0

In the case of readings taken in March, 1920, no marked difference in amount of citrus blast was detectable upon general observation. Two blocks of nine trees each in a third orchard, which were sprayed with ammoniacal copper carbonate (3-6-50) and Ortho lime-sulfur (three per cent) respectively on December 16, 1919, showed no results which might help in controlling blast.

The second year, 1920-1921, a series of experiments were carried out as indicated in table 13. These were made on trees similar to those of table 11, which were by this time six years of age.

TABLE 13  
NUMBER OF AFFECTED LESIONS PER TREE

No. of trees	Treatment	Number of lesions			Ratio of number of lesions on south side to that on north
		Dec. 16	Jan. 25	Feb. 16	
10	Bordeaux, Nov. 2.....	2.5	65	329	6
22	Bordeaux, Dec. 16.....		139	395	3
11	Bordeaux, Nov. 2 and Dec. 16.....	2.8	71	233	5
11	Am. CuCO <sub>3</sub> , Nov. 2.....	5.0	139	440	3
11 {	Bordeaux, Dec. 16, and Am. CuCO <sub>3</sub> , Nov. 2..... }	3.8	173	515	3.5
19	Check, east side of grove.....	18.2	262	658	3
13	Check, west side of grove.....	27.8	250	771	2.7

It will be noted that in this record the individual lesions, instead of the affected twigs, were counted. As in the case of the previous year, the figures indicate only partially the difference between the sprayed and unsprayed trees, as the lesions on the sprayed trees

appeared to average less than those on the unsprayed trees and therefore fewer branches were killed. The best results were obtained from the two plots sprayed on November 2. The plot which received a second spraying on December 16 appeared only slightly improved, and where the spraying was delayed till December 16 the benefit was still less. The results of the spraying with ammoniacal copper carbonate indicate that much less benefit was received in that case than from that with the Bordeaux.

The relative difference in number of lesions between the north and south sides of the trees, the reasons for which have already been discussed, was an interesting feature. On the unsprayed trees there were approximately one third as many lesions on the north as on the south side. On the trees sprayed with Bordeaux on November 2 there were one fifth and one sixth as many on the north as on the south side. The prevention, therefore, appeared to be relatively more effective on the north than on the south side. Possibly the driving rains from the south in the Oroville section would tend to lessen the relative efficiency on the south side. It is believed that these counts are fairly representative, as several tests in which counting was done from individual trees by different observers or repetitions which were made by the same observer showed that the variation in repeated counts was small.

During the third year, 1921-1922, several tests were made by growers. These varied as to time of application. The results point to the same conclusions regarding the importance of early fall spraying.

One grower sprayed part of an orchard of navel orange trees with Bordeaux (5-5-50) on November 1st to 3rd, 1921. About April 1, 1922, an examination of the trees sprayed showed almost no blast on them, while unsprayed adjacent trees were badly affected. A second block was sprayed November 28, 1921, but the results were not as satisfactory. On representative samples from these trees only one lesion to several hundred leaves was found on the twigs sprayed about November 1, while 10 to 25 per cent was the average number with twigs sprayed November 28, and about 50 per cent with twigs not sprayed.

Another grower sprayed with Bordeaux December 5, 1921, and although the prevention was not as effective as that which resulted in

the case above, the benefit appeared marked when comparison was made with contiguous unsprayed trees. Other sprayings made from December 10th to 15th showed differences detectable upon observation, but not sufficiently marked to be commercially successful.

The results of the experiments made in these three years all point to the conclusion that an early fall spraying (about November 1) is more efficient for prevention of citrus blast than later ones, even though the activity of the blast apparently seldom begins before January. The reason for this fact is not known.

### RECOMMENDATIONS

1. Spray with Bordeaux mixture as early as November 1. A second spraying on or before December 15 may be helpful, but the first alone is much more effective than the second alone. The spraying should be thorough in order to cover the entire surfaces of the twigs and leaves. It is therefore suggested that a spreader such as casein be added.

2. When it is possible, grow a tree with a bushy, compact type of growth, having a minimum liability to injury during severe wind storms.

3. In the case of new planting of citrus provide some means of protection against injury caused by the south winds.

These suggestions are of little importance for conditions in southern California, since the disease is a minor one there, usually manifesting itself only in black pit of the fruit which is seldom of sufficient importance to demand a remedy. Spraying trees with Bordeaux would be detrimental in places where fumigation with cyanid is practiced. With regard to protection against injuries the same principles apply to all sections.

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## PLATE 1

Citrus blast lesions on leaves and twigs of Navel orange.

Fig. A. Active lesions starting in February from infection at a tear caused by the wind on the wing of the petiole. Points of infection indicated by the arrows.

Figs. B and C. Inactive lesions of the previous year on live twigs, showing by the size and condition of scabs some of the different degrees of severity in the development of the disease. B shows an average condition at Oroville while C shows a very severe development.









## PLATE 2

Showing small lesions surrounding attached petioles. These have attained their maximum development at the end of the active period. Near the center is a typical example of the many dead dry leaves which frequently remain firmly attached through the subsequent summer and fall.





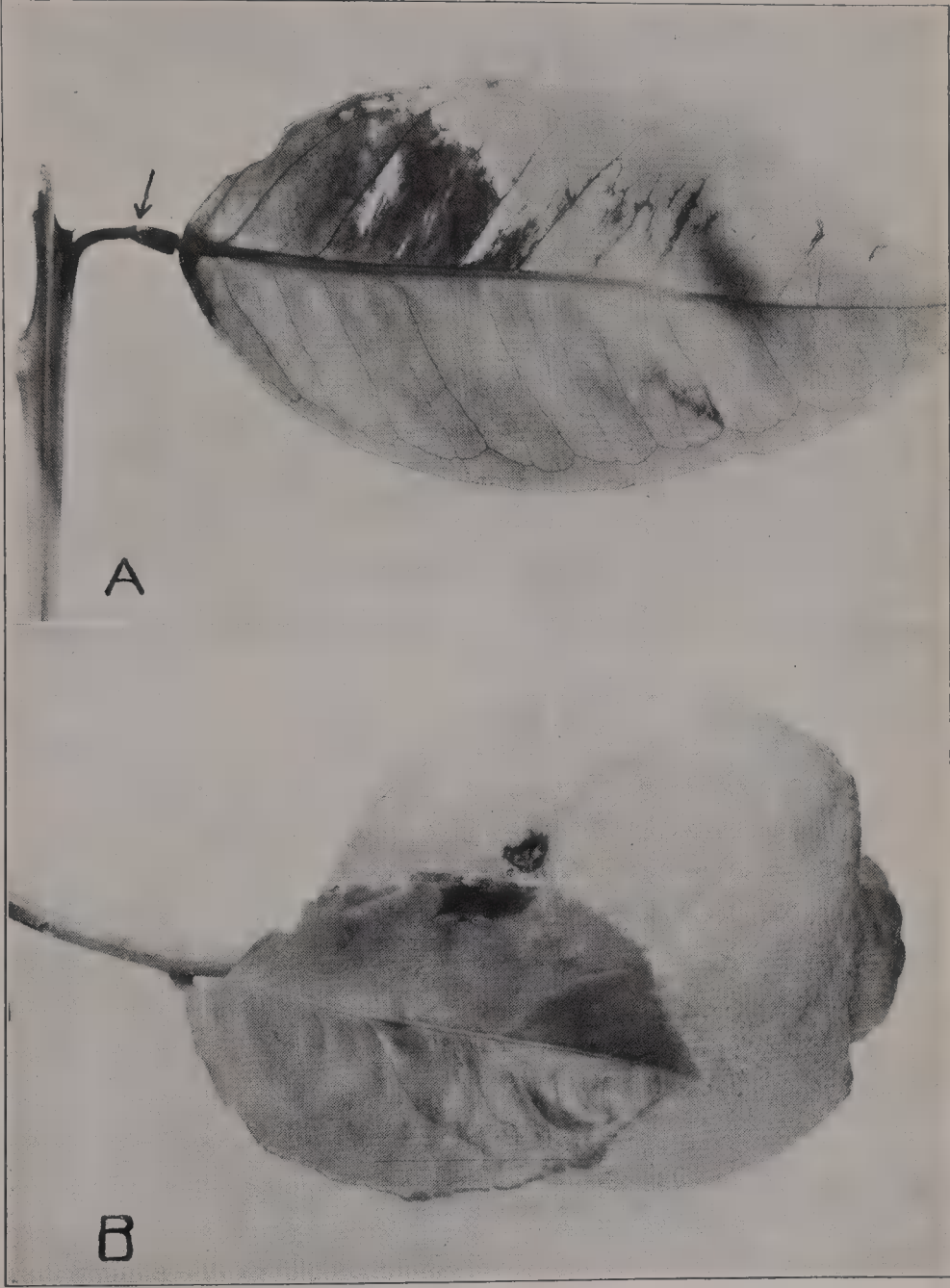


### PLATE 3

Fig. A. Blast on stem and leaf of Navel orange. The arrow points to the tear in the wing of the petiole where infection probably started and spread in both directions to the leaf blade and to the twig which is being killed back. A surface stain or scorch has also spread out over one portion of the leaf blade.

Fig. B. Blast on the leaf blade and black pit next to it on the fruit of the lemon.









#### PLATE 4

Black pit spots developing from natural infections in slight injuries to fruit, Oroville, California, February, 1921. *Pseudomonas citriputale* was isolated from some of these lesions in each species. A, Navel orange. B, Grapefruit. C, Three lemons. On several of the spots slight scratches which opened the way for infection are shown.

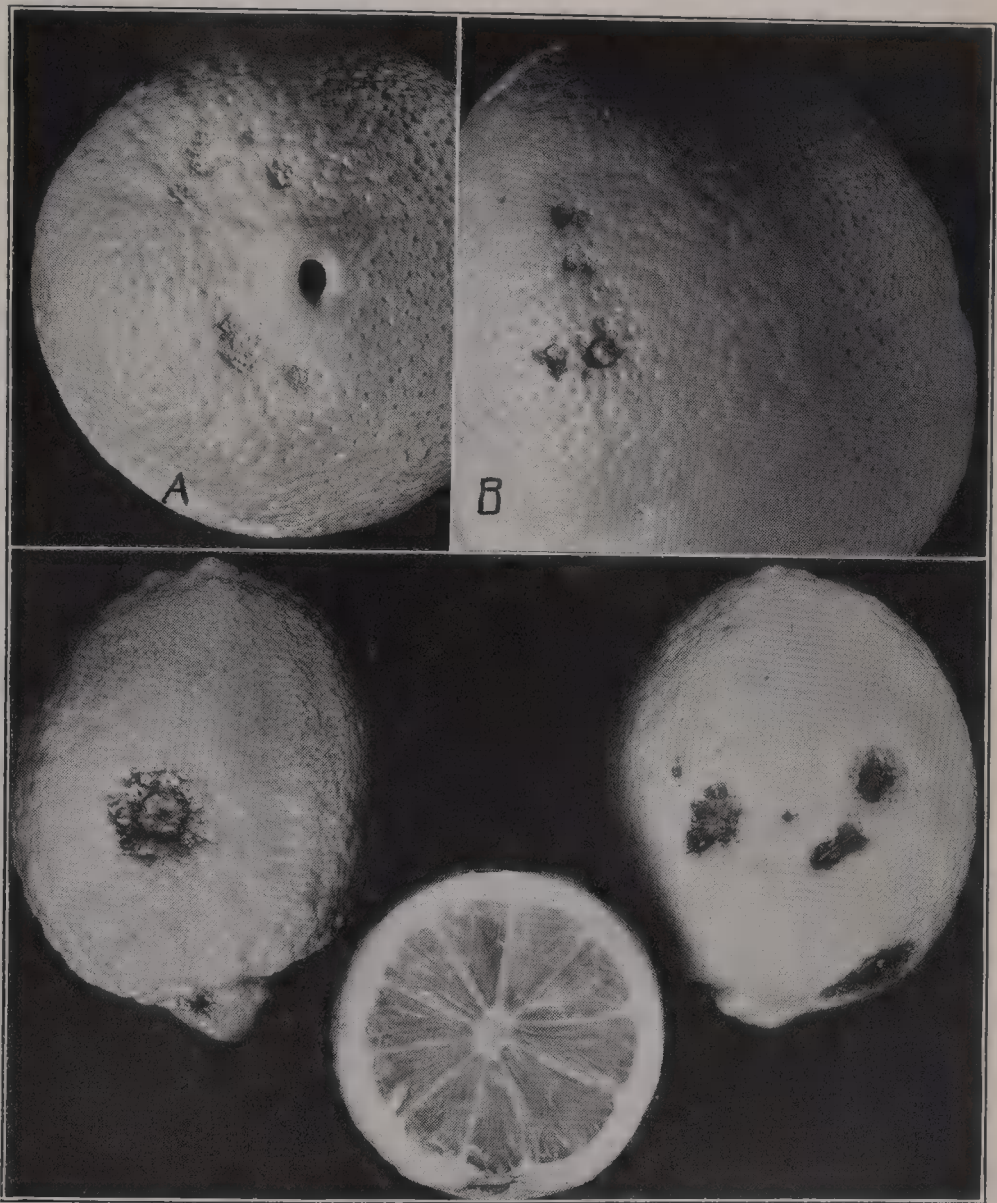


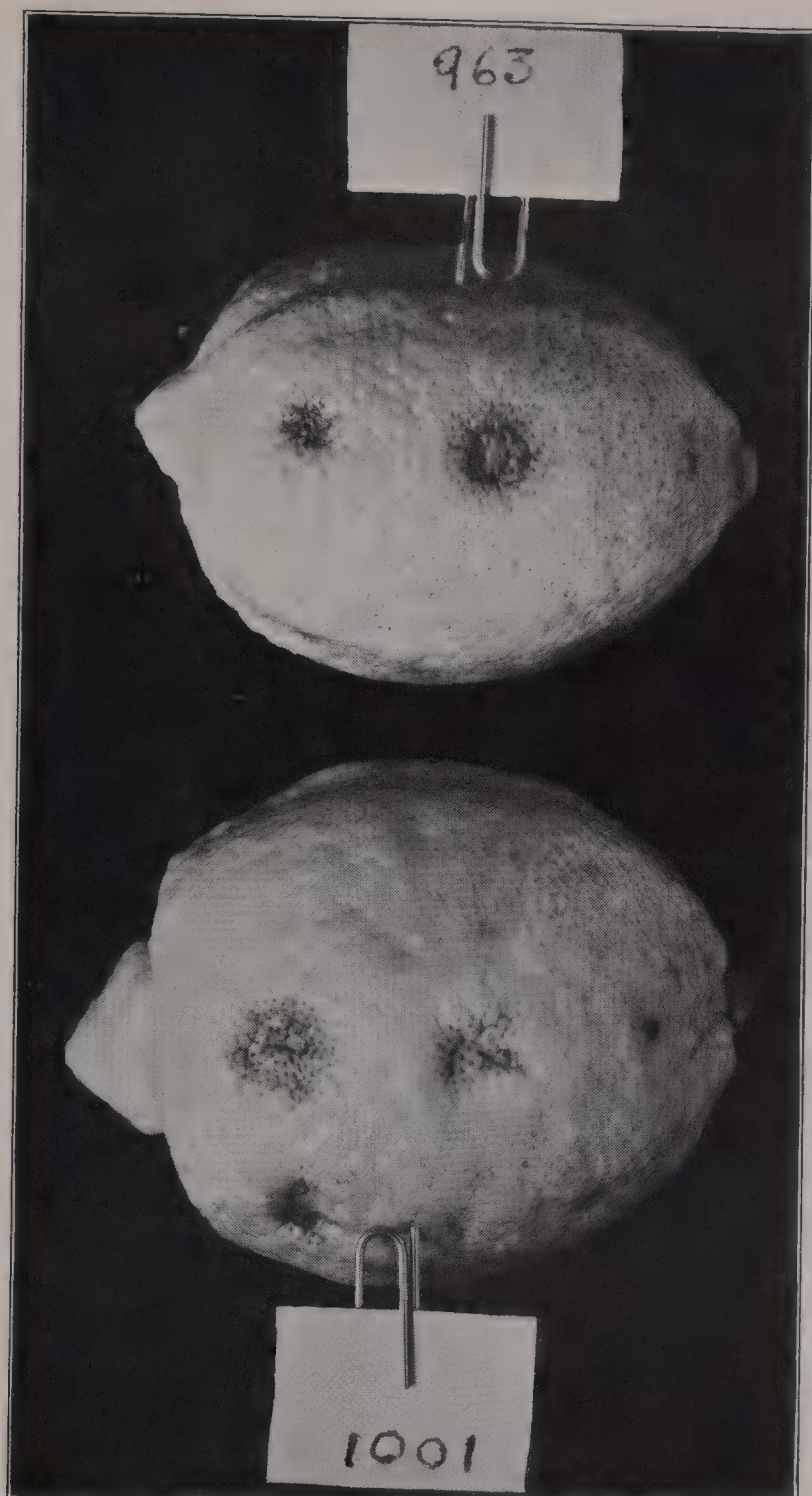






PLATE 5

963 shows black pit spots on a lemon grown near Alta Loma in southern California. The spots shown on 1001 were produced by inoculating with a culture isolated from a citrus blast lesion on a Navel orange twig grown near Oroville in northern California.



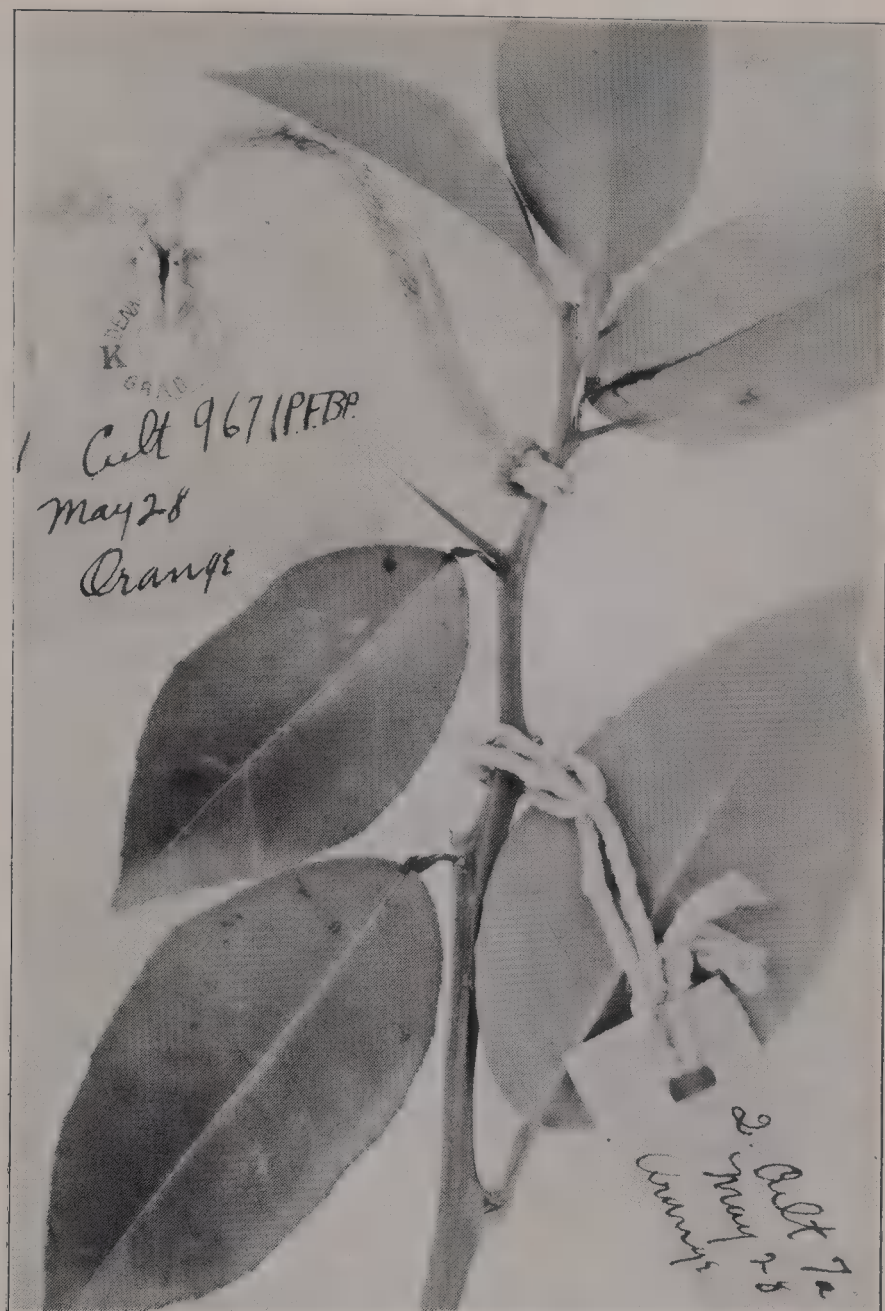




#### PLATE 6

Three citrus blast lesions on orange leaves. Two upper lesions produced by inoculations with culture of *Pseudomonas citriputeale* isolated from black pit spots on lemons grown in southern California, and lower by culture isolated from citrus blast lesion on orange twig grown in northern California.







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\* Abridged from thesis submitted in partial fulfilment of requirement for the degree of Master of Science, University of California, May, 1921.

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Horticulturists have at various times devoted considerable thought and study to the question of rootstocks. The problem is of peculiar interest to the orchardist and nurseryman of California on account of the wide range of soil and climatic conditions existing within the confines of the state, bringing into existence the most extensive and varied fruit industry of the world. On account of this diversity the question of proper rootstocks for different fruits and various environments here has assumed an importance not approached in any other fruit growing region.

The success of fruit raising in California, which has been of the greatest commercial importance for more than half a century, has been due more to the rich soils and favorable climatic environments of the state than to the expert knowledge of the growers. With the advent of higher land values and keener competition, orchardists are each year demanding more specific knowledge of the facts upon which their business is based. As the acreage devoted to horticultural crops has increased in California, plant pests—insects, bacterial, and fungoid—have become more numerous both by introduction from other regions and by adaptation from native host plants. Accumulated experience and observation lead the present-day horticulturist to the conclusion that in order to secure rootstocks adapted to various environments, which at the same time are resistant to plant pests, it will be necessary to make a thorough study of the whole problem.

In recent years excessive irrigation has caused the water-table to rise in certain orchard regions to such an extent that the root systems are often submerged in water for long periods of time. As virgin soils are put under cultivation new problems constantly arise; for example, the calcareous nature of the subsoil in one section is causing fruit growers considerable apprehension. The leaves of pear trees are there rendered chlorotic by the excessive lime content of the soil. Observations have shown that trees on certain stocks are more resistant to chlorosis (which seems to be physiological in nature), than those on other stocks. Other problems facing the present-day grower

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are pear blight (*Bacillus amylovorus*) and oak root fungus (*Armillaria mellea*), diseases for which no satisfactory methods of control have yet been discovered. The remedy for the above mentioned troubles will possibly be found in the selection of proper rootstocks. Native untried roots may solve many of these problems, but should this not prove to be the case, desirable roots indigenous to other countries must be found and utilized.

One of the chief difficulties the California Agricultural Experiment Station has encountered in conducting a systematic study of the rootstock problem, has been the lack of a definite means of identification of roots of deciduous fruit trees now in common use. In the hope that a more definite knowledge of the subject might be had, this investigation was undertaken with two objects in view:

1. To determine, if possible, whether constant gross morphological differences exist between the more common rootstocks used for the propagation of deciduous fruits in California, which may be used as means of identifying the species when nothing more than a portion of the root system is available.

2. To differentiate between the various kinds of stocks on the basis of microscopic anatomical study of their root structure.

## SURVEY OF LITERATURE

Burgerstein<sup>3</sup> made a study of the wood (trunk and stem) of the Pomaceae and Amygdalaceae, including several species that were used in the present investigation. He worked chiefly with cell measurements as follows:

- a. The radial width of the tracheae.
- b. The radial thickness (lumina and walls) of the tracheae in the spring wood.
- c. The radial width of the wood parenchyma cells.
- d. The height of the medullary ray cells.
- e. The width of the medullary ray cells.

Although Burgerstein did not study the roots of the trees, his findings are of interest because he showed that little variation exists in the points investigated between different species. The following

is a portion of one of the many tables presented by Burgerstein, all of which are similar in nature:

Species	Width of tracheae	Height of medullary rays	Rows of cells in medullary rays
<i>Prunus Amygdalus</i>	.080-.120 mm.	.019 mm.	1-5
<i>Prunus Armeniaca</i>	.056-.070 mm.	.021 mm.	1-5
<i>Prunus Persica</i>	.080-.107 mm.	.024 mm.	1-4
<i>Prunus Mahaleb</i>	.053-.083 mm.	.018 mm.	1-4

It can readily be seen that data such as the above, if used alone, would be of little value in identification work.

Although Burgerstein's work with cell measurements gives little additional information on the present problem, his investigations on the differences between Amygdalaceae (*Prunus*) and Pomaceae (*Pyrus* and *Cydonia*) species are of great value.<sup>4</sup> He summarizes these differences as follows:

POMACEAE	AMYGDALACEAE
Tracheae single.	Tracheae single or in groups of 2-5.
Tracheae egg-shaped or elliptical, very seldom circular.	Tracheae circular, elliptical, oblong or irregular.
Height of medullary rays 0.5 mm.	Height of medullary rays up to 1.4 mm.
Medullary rays 1-3 cells in width, mostly 1-2	Medullary rays 1-10 cells in width, mostly 1-4.

In referring to the above table Burgerstein states "... the woody structure of Amygdalaceae and Pomaceae reveal no essential or outstanding differences."

Hanausek<sup>5</sup> states, "The *Prunus* woods are much alike and difficult to distinguish." He further declares, "They [*Prunus* woods] are distinguished from similar woods of the Pomes by the pore ring [ring of tracheae in each annual ring] which is absent in the latter." Hanausek also states, "The wood of the almond is very similar to that of the apricot." On the whole, Hanausek's work indicates that the woods of the species of the genus *Prunus* are much alike.

Solereder<sup>8</sup> summarizes the histological characters of the Rosaceae under the following headings:

- a. Anatomical features.
- b. Leaf structure.
- c. Oxalate of lime content.
- d. Secretory organs.
- e. Hairy covering.
- f. Petiole.
- g. Pith, etc.

He states that: "Constant anatomical characters are almost wanting in this Order." Also, "... cork formation is superficial in Prunae and Pomeae." He further states, "With regard to the structure of the root, it may be mentioned that in many genera the layer of cells situated immediately outside the endodermis is provided with ridge-like thickenings on the radial and transverse walls (Van Tieghem's 'reseau sus-endodermique'). These are found in Prunus, Pyrus and Cydonia." In referring to the wood Solereder says: "Among the Pomeae distinct spiral thickenings of the walls of the vessels are found in . . . Cydonia, . . . and are wanting in Pyrus. The wood in all the Pomeae contains scattered vessels which in the course of each annual ring show a gradual decrease in size. With reference to the structure of the wood in Prunus, . . . in all the investigated species, the vessels show a spiral thickening band . . ." Solereder's work indicates that a close relation exists between the species of Prunus and of Pyrus.

In his discussion of the structure of the roots of the Dicotyledons Solereder says:

"The structure of the roots in the Orders of the Dicotyledons has not been methodically investigated to any considerable extent, because the requisite material is generally wanting, and the anatomical investigation of the leaf and axis, which are more easily obtained and show a greater diversity of structural features, still affords abundant scope for research. The more important characters to be taken into account in an investigation of the root are as follows:

"a. The number of xylem and phloem groups in the primary fibro-vascular system.

"b. The mode of differentiation of the secondary xylem-mass of the wood being either, (1) unlignified, and in this case containing vessels only or vessels together with scattered groups of wood-fibres, or (2) lignified, in which case it is composed for the most part of wood-fibres; these features are connected with the physiological functions of the root (as a nutritive or attacking organ, as a respiratory root, etc.) and are therefore subject to variation within certain limits in one and the same species.

"c. The occurrence of peculiar ridge-like or otherwise-shaped thickenings (Van Tieghem's 'reseau de soutien') in the sub-epidermal layer of cells or in the more deeply situated cells of the primary cortex."

Regarding the number of xylem and phloem groups referred to by Solereder, Van Tieghem<sup>9</sup> says, "... in Prunus and Pyrus there are four groups, in Amygdalus (Almond) six groups." In another

report<sup>10</sup> he states, "A strengthening net-work (reseau de soutien) has been observed in the Rosaceae."

Bastin<sup>2</sup> gives a report on the structure of the stem barks of our native cherries. In his paper he discusses the two species of cherries studied in the present investigation, namely *Prunus avium* and *Prunus Mahaleb*. Referring to the Mahaleb he says, "The sclerenchymatous elements consist mostly of small clusters of bast fibres, stone cells being wholly or nearly absent. The ducts (tracheae) are most abundant and largest adjacent to and on the exterior side of the ring of growth, so that the rings of growth are rendered much more conspicuous to the eye." Regarding *Prunus avium*, he says: ". . . it differs from the other species in the decidedly fibrous character of the bast layer. There are not only scattered and very tortuous lignified fibres in its outer portions, or even extending into the middle bark, but clusters and isolated fibres occur abundantly throughout the bast. The masses of bast fibres are never very large and are not arranged with any apparent order. The taste of this bark is decidedly bitter and astringent."

Bastin summarizes some of his observations in the following manner:

"Structurally the barks of the different species of cherries examined resemble one another in the following particulars:

"1. The phellogen [cork] begins its formation in the layer of collenchyma cells beneath the epidermis, and no considerable development of the phelloderm [green parenchyma] layer beneath takes place.

"2. They all resemble one another in the facility with which the periderm [cork cambium and the derivative tissue] layers are separated transversely into thin sheets.

"3. The medullary rays in all the species are several rows of cells thick, though the number of cells differ somewhat in the different species.

"4. In all the barks examined the medullary rays are more or less wavy in their course, though less so in some species than in others.

"5. All the barks show a strong tendency to fissure between the medullary rays and adjacent bast tissues.

"6. All the barks possess a bitter, astringent and more or less aromatic taste, but the bitterness is much less marked in some than in others, and the aromatic quality is very decided in some, but barely perceptible in others.

"The most prominent structural differences are in the number, arrangement and character of the sclerenchymatous elements."

Taken as a whole the preceding review of available literature would seem to indicate that the identification of the various deciduous fruit rootstocks by morphological or histological characters would be practically impossible. As has been observed, several workers have shown the degree of similarity that exists between the various species of woods. Although most of these writers worked with the branches and trunks, their investigations brought out clearly the fact that separation of the various roots by anatomical characters would probably be very difficult, if not altogether impossible.

With the hope of obtaining viewpoints, not included in the literature above cited, letters of inquiry were sent to botanists, horticulturists and nurserymen in various parts of the United States, asking for their opinion as to the possibility of being able to tell the different species of rootstocks apart when only small portions of the roots were available for study. The consensus of opinion was that, owing to the close botanical relationships existing between the various rootstocks, there are probably no definite morphological or histological characters that would be of value for identification.

### MATERIAL USED IN THE INVESTIGATION

The following list includes all the roots used in the investigation:

#### Genus *Prunus*

<i>Prunus Armeniaca</i> Linn. ....	Apricot
<i>Prunus Persica</i> S. & Z. ....	Peach
<i>Prunus Amygdalus</i> Stokes .....	Almond
<i>Prunus cerasifera</i> Ehrh. ....	Myrobalan plum
<i>Prunus avium</i> Linn. ....	Mazzard cherry
<i>Prunus Mahaleb</i> Linn. ....	Mahaleb cherry
<i>Prunus Davidiana</i> Franch .....	Davidiana peach

#### Genus *Pyrus*\*

<i>Pyrus serotina</i> Rehd. ....	Japanese pear
<i>Pyrus communis</i> Linn. ....	French pear
<i>Pyrus ussuriensis</i> Maxim. ....	Ussuriensis pear
<i>Pyrus Calleryana</i> Decne. ....	Calleryana pear
<i>Pyrus Malus</i> Linn. ....	Apple

#### Genus *Cydonia*\*

<i>Cydonia oblonga</i> Mill. var. Orange .....	Orange quince
<i>Cydonia oblonga</i> Mill. var. Angers .....	Angers quince

\* Owing to the degree of similarity found to exist between the genus *Pyrus* and the genus *Cydonia*, the word "Pyrus" used hereafter will, unless otherwise stated, refer to both genera. (See pages 20 and 21.)



The above list includes practically all the deciduous fruit rootstocks that are of commercial importance in California. The Paradise and Doucin apples are omitted, as material for study was not available. These are simply types of *Pyrus Malus* and hence would possibly show no appreciable differences.

Since ecological factors cause certain quantitative and qualitative differences in many kinds of plants, it was thought possible that such diversities might well extend to the root systems of fruit trees. It could not be expected that the size, shape and general character of a single cell, or group of cells, would be identical under widely varying conditions of soil, climate and cultural methods. Why should a French pear root grown with an abundant moisture supply, as along the Sacramento River, be exactly like one grown under the arid conditions of the Antelope Valley? In order to study the results of such influences, specimens of as many species as possible were secured from the following sources:

University Farm, Davis, California.

U. S. Plant Introduction Gardens, Chico, California.

Santa Clara Valley, California.

Southern Oregon Agricultural Experiment Station, Talent, Oregon.

Austin Nursery, Austin, Texas.

Orengo Nursery, Orengo, Oregon.

Stark Brothers Nursery, Louisiana, Missouri.

## METHODS

Two distinct lines of procedure were followed:

*a.* Field methods.

*b.* Laboratory methods.

*Field Methods.*—All field work was carried on in the orchards of the University Farm at Davis, California. Roots were selected with three points in mind: To obtain specimens, first, from trees varying in age; second, growing in different soil types; and third, from different nurseries. One of the main or subsidiary roots was exposed and then closely examined for gross morphological characters, such as lenticels, color, striations on bark, or habit of branching. The root was generally severed from the tree and taken together with some



suckers, for identification purposes, to the laboratory for further examination. In all cases the name given to the roots was verified by an examination of the leaves of the suckers. Bailey's<sup>1</sup> "Cyclopedia of Horticulture" proved of great value in the identification by above-ground characters. After a thorough examination had been made of the external characters, a horizontal cut was made through the root, exposing the wood structure in a transverse view. By aid of a hand lens the tracheal vessels, medullary rays and cortex were then studied.

*Laboratory Methods.*—The methods used in the laboratory consisted of preparing and studying paraffin and free-hand sections. The technique used in preparing and staining the sections was essentially that outlined by Chamberlain.<sup>5</sup> Delafield's haematoxylin and safranin gave the best results for staining. Owing to the hardness of the roots, it was found desirable to employ some method of softening in order to facilitate subsequent cutting. Very good results were obtained by using hydrofluoric acid in the manner described by Jeffrey.<sup>7</sup> When it was desirable to preserve fresh material for future study, the roots, when large, were wrapped in damp moss and kept at a temperature of 32° F. By first dipping the roots in a dilute formaldehyde solution, the beginning of mold growth was materially delayed. Portions of small roots were preserved in solution. This form of preservation must be used when cold-storage facilities are not available. Roots may be kept indefinitely without any marked injury in the following solution:

Commercial formaldehyde (40 per cent).....	2 cc.
60 per cent ethyl alcohol .....	98 cc.

In studying transverse sections careful observations were made of the following:

Bast fibres:

- a. Number and arrangement; whether in irregular groups or in regular concentric rings.

Cork cells:

- a. Ratio between width and length.

Medullary rays:

- a. Whether terminated in the center of the cortex or near the cork cambium.
- b. Whether broadened out as they entered the cortex or of the same width as in the sap-wood.

- c. Length and width of the cells.
- d. Tangential width in cell number.

Pits:

- a. Character of pitting in cell walls of all cells.

Sclerides:

- a. Number and position.

Star in center of root:

- a. Presence or absence.
- b. If present, number of points in it.
- c. Type of cells making up star; whether large, loose cells or small compact ones.

Tracheae:

- a. Size, arrangement and number.
- b. Arrangement of tracheae in spring wood of annual rings.
- c. Tracheae single or in groups of two or more.

Wood fibres and wood parenchyma:

- a. Types of cells bordering the medullary rays; whether wood fibres or wood parenchyma cells.
- b. Presence or absence of wood parenchyma.
- c. Ratio between the amount of wood fibres and wood parenchyma.
- d. Size and shape of wood fibres.
- e. Arrangement of wood fibres; whether isolated, or in radial or tangential groups.
- f. Whether wood parenchyma was metatracheal (tangential bands) or paratracheal (aggregated around the tracheae).

Miscellaneous:

- a. Difference in width of cell walls of tracheae, wood fibres and wood parenchyma.
- b. Variations in size of cells in spring and fall wood.

In the longitudinal sections special study was made of the following features:

Bast fibres:

- a. Length.

Cork cells:

- a. Height.

Medullary rays:

- a. Height of cells in millimeters and in number of cells.

Pits:

- a. Presence or absence of bordered pits in cells of different tissues.

- b. Arrangement and size of pits in cells of various tissues.
- c. Angle made by the slit of the pit with the vertical axis of the root.

Star:

- a. Type or appearance of cells making up star.

Tracheae:

- a. Distance in millimeters between broken down walls (cross-walls) in tracheae.
- b. Type of thickening on the walls of the tracheae; whether spiral, scalariform, reticulate, etc.

Wood fibres and wood parenchyma:

- a. Length of wood fibres in millimeters.
- b. Length of wood parenchyma cells in millimeters.
- c. Type of walls in wood parenchyma cells; whether horizontal or oblique.

By using the above criteria and by examining a large number of sections, many outstanding characteristics were found that are of value for identification. Although most of the distinguishing features can only be seen with a microscope, many of them may be observed with a hand lens.

As a result of the studies made, the writer has been able to detect many slight variations more or less constant in nature, which are most difficult to describe and practically impossible to illustrate. Only those characteristics which may be easily interpreted have been used in the discussion of the histological features of each species. In general, all these features have been found susceptible of illustration.

## PRESENTATION OF DATA

One of the interesting points brought out by this investigation is the degree of similarity, from a histological point of view, between certain species of roots whose above-ground characters are strikingly different. Although outstanding differences exist between some roots, the reader will note that in many cases these diversities are indefinite and cannot be used with absolute assurance for identification. Despite the fact that similarities between the different species are of utmost importance, greater stress will be placed on their differences, for it is the chief aim of this paper to present the latter. In order to present the data in a clear manner, a key will be used as the basis for discussion, accompanied by photographs and tables.

# KEY FOR THE IDENTIFICATION OF THE COMMONLY USED DECIDUOUS FRUIT TREE ROOTSTOCKS

A. Medullary rays 1-10 cells in width, usually 5-8; sides of tracheal vessels never angular but always smooth and more or less circular in outline; heart and sap wood practically free from wood parenchyma.

I. Cells directly beneath cork layer usually free from calcium oxalate crystals; ray extensions in cortex not sinuous .....

.....*Prunus*, except the cherries

B. Bark beet-red in color.....*Prunus Armeniaca*

BB. Bark other than beet-red in color.

a. Lenticels large and prominent on roots of all sizes, protruding one-eighth of an inch or more above surface of bark and reaching a longitudinal height of one-eighth of an inch or over, slit very indefinite and surface of lenticel very rough; bark always glossy and smooth; young roots yellow in color, becoming darker as the root increases in size; cortex relatively thick.

x. Tracheae joined in groups of 2-5 cells each .....

.....*Prunus Davidiana*

xx. Tracheae single, vessels being distributed more or less evenly throughout the entire wood mass; first vessels formed in each annual ring circular in cross-sectional view.....*Prunus Persica*

aa. Lenticels small on young roots, never protruding more than one-sixteenth of an inch above surface of bark on old roots; bark never glossy but usually rough; cortex relatively thin.

x. Wood parenchyma practically lacking; radial width of medullary ray cells equal to or slightly greater than tangential width; young roots yellow, old roots brown in color; tracheae diminishing rapidly in cross-sectional size as the fall wood is approached; first vessels formed in each annual ring elongated in cross-sectional view.....*Prunus Amygdalus*

xx. Wood parenchyma cells usually present in large numbers; radial width of medullary ray cells usually 2-5 times that of tangential width; medullary rays rarely exceeding five cells in width; brown usually the dominating color .....

.....*Prunus cerasifera*

II. Cells directly beneath cork layer containing calcium oxalate crystals; ray extensions in cortex sinuous .....

.....Cherries (*Prunus avium* and *Prunus Mahaleb*)

B. Taste bitter and astringent; tracheae generally scattered uniformly throughout the entire wood mass; lumina of wood fibres two or three times as wide as wall thickness; calcium oxalate content of collenchyma cells small .....

.....*Prunus avium*

- BB. Taste not bitter and astringent; tracheae usually confined to early growth of each year; lumina of wood fibres equal to or generally less than thickness of cell wall; calcium oxalate content very high; in many cases all the collenchyma cells possessing crystals ..... *Prunus Mahaleb*
- AA.\* Medullary rays one or two cells in width, rarely three, sides of tracheal vessels angular, usually 6-9 sided; heart and sap wood possessing numerous wood parenchyma cells..... *Pyrus* (including *Cydonia*)
- I. Cells of cortex large, exhibiting an open, loose structure..... *Pyrus Malus*
- II. Cells of cortex small and compact in arrangement.
- B. Lumina of wood fibres equal to or slightly greater in width than diameter of cell wall..... *Cydonia oblonga*
- BB. Lumina of wood fibres several times as wide as wall thickness.
- a. Vessels in spring wood of each year appear slightly elongated in transverse section, walls slightly angular ..... *Pyrus serotina*
- aa. Vessels in spring wood of each year appear nearly isodiametric in transverse section; walls very angular ..... *Pyrus communis*

An examination of the key reveals the fact that it is really divided into three distinct groups: first, *Prunus*, except the cherries; second, the cherries; and third, *Pyrus*, including *Cydonia*. For purposes of discussion, each group will be taken up individually, preceded by a description of the distinguishing features of the genera, *Prunus* and *Pyrus*.

#### SEPARATION OF PRUNUS AND PYRUS (INCLUDING CYDONIA) SPECIES

These genera may be distinguished by any one of the three characters mentioned in the key. The variations are somewhat comparable to those Burgerstein<sup>4</sup> noted in the stem woods of the two genera. It will be recalled that his differentiation was based on the shape of the tracheae and the height and width of the medullary rays. (See page 4.)

The roots in all the species of *Prunus* have medullary rays that are many cells in width. This is especially true in old roots, but they may also be found in young roots as small as one thirty-second of an inch in diameter. The medullary rays of the *Pyrus* roots, on the other hand, are practically always only one or two cells in width,

\* *Ussuriensis* and *Calleryana* pears are not included in this key, owing to the indefiniteness of their differentiating features. (See pages 22 and 23.)



regardless of the size or age of the root. This variation is quite constant and may be relied upon in distinguishing *Prunus* from *Pyrus* species (pl. 1, figs. 1 and 2).

The shape or outline of the tracheae, as seen in cross-section, may also be used for identification. In all *Prunus* species these vessels are more or less circular in outline and their walls are quite smooth and continuous. On the other hand, the *Pyrus* species consistently exhibit an angular and irregular wall (pl. 1, figs. 1 and 2).

The third means of identification suggested is the general histological nature of the heart and sap wood. In all species of *Prunus* they are composed almost entirely of wood fibres. In species of *Pyrus* the wood consists largely of wood parenchyma (pl. 1, figs. 1 and 2).

In determining whether a root is *Prunus* or *Pyrus*, all three or any one of the above features may be used for identification. In cases of doubt, all three should be studied, but it is unlikely that any difficulty will arise with the outstanding differences existing.

#### GROUP I. PRUNUS SPECIES, EXCEPT THE CHERRIES

<i>Prunus Armeniaca</i> Linn.....	Apricot
<i>Prunus Davidiana</i> Franch.....	Davidiana peach
<i>Prunus Persica</i> S. & Z.....	Peach
<i>Prunus Amygdalus</i> Stokes.....	Almond
<i>Prunus cerasifera</i> Ehrh.....	Myrobalan plum

#### *Prunus Armeniaca*, Apricot

By observing the key it will be noted that surface color divides the above group into two parts, with *Prunus Armeniaca* forming one, and *P. Davidiana*, *P. Persica*, *P. Amygdalus* and *P. cerasifera* the other. The beet-red color of *Prunus Armeniaca* is definite enough to allow of positive identification without resorting to further study of any other characters. However, the following specific structural features are given for the purpose of comparison with other species of *Prunus*.

Plate 1, figure 3, shows the characteristic barrel-shaped cells that make up the medullary rays of the apricot root. As a rule, the tangential diameter of the cells is about the same, or a little less, as is the radial diameter.



As is shown in plate 1, figure 4, the rays start to spread and become fan-shaped when they reach a point mid-way between the cambium and surface of the bark.

In longitudinal radial section the cork cells appear small and regular in size, while in transverse section they are wide tangentially and narrow radially. The following gives the average measurements of the cells:

Longitudinal height (longitudinal section).....	.014 mm.
Tangential width (transverse section).....	.050 mm.

### *Prunus Davidiana* and *P. Persica*, Peaches

These two species of *Prunus* differ from *Prunus Armeniaca* in that their bark color is yellow rather than red, especially while the roots are small. In many respects the two species are identical, especially in surface color, lenticel characters and thickness of cortex. As the roots become old and increase in size, the color assumed is somewhat reddish. This color, however, is strikingly different from that characteristic of *Prunus Armeniaca*, in that it is a dull, deep red, rather than the bright, beet-red of the latter.

The bark of peach roots is rather smooth and always appears glossy. It also exhibits a character that seems to be characteristic, viz., that when the root dries out, lateral and sometimes longitudinal cracks appear. These are very conspicuous, as is seen in plate 2, figure 1.

The cortex of peach roots is relatively thick. The word "thick" cannot be definitely described but in the sense here used means that roots up to one and one-half inches in diameter have a cortex that averages about one-eighth inch in thickness (pl. 2, fig. 2).

Regardless of the size of the peach root, the lenticels always appear large and prominent. On roots over one and one-half inches in diameter the lenticels protrude one-eighth inch or more above the surface of the bark and reach a longitudinal height (distance measured parallel to axis of root) of one-eighth inch or over. Owing to the rough surface, the slit of the lenticel is not clearly defined (pl. 2, fig. 1).

*Prunus Davidiana* and *Prunus Persica* are indistinguishable macroscopically. Their chief point of differentiation lies in the character of the tracheae is seen in cross-section. Plate 2, figure 3, shows

the characteristic grouping of the tracheae in *Prunus Davidiana*. This grouping is typical of *Prunus Davidiana*, but not so with *Prunus Persica*. In the latter the vessels are arranged singly.

Plate 3, figure 1, shows the characteristic distribution of the tracheae in *Prunus Persica*. It will be noted that the vessels are distributed evenly and also that they diminish gradually in size as the fall wood is approached. The first vessels formed in each annual ring are circular in outline, when seen in transverse section, and attain a radial diameter of about .12 mm. Exclusive of the first vessels formed in the annual rings of *Prunus Davidiana*, the tracheae are small when compared with those of *Prunus Persica*. This is clearly shown by the following table:

Species	Average radial diameter of tracheae
<i>Prunus Persica</i> .....	.096 mm.
<i>Prunus Davidiana</i> .....	.066 mm.

In *Prunus Persica* the medullary rays assume the shape of a fan after reaching the inner portion of the cortex (pl. 3, fig. 2). *Prunus Davidiana* does not exhibit the fan-shaped ray extension; instead the rays keep their same width and merge into the surrounding tissue, as is shown in plate III, figure 3. There are, however, a few exceptions to this statement.

#### *Prunus Amygdalus*, Almond

In color, *Prunus Amygdalus* is somewhat similar to *Prunus Persica* and *Prunus Davidiana*; instead of being of a bright glossy yellow its color is usually dull yellow, changing to a dull brown as the root becomes older. The surface of the bark is decidedly rough.

The chief differentiating feature of *Prunus Amygdalus* is the character of the lenticels. While protruding one-eighth inch or more above the surface on peach roots, the lenticels on *Prunus Amygdalus* rarely extend more than one-sixteenth of an inch above the bark surface. The lenticels are few in number, comparatively smooth and the slits are very distinct. As a rule, the longitudinal height of these organs never exceeds one-sixteenth of an inch (pl. 2, fig. 1).

The cortex of this root is relatively thin. On roots two inches in diameter the thickness of the cortex rarely exceeds one-sixteenth of an inch, one-half that of the peach cortex. This is clearly shown in plate 2, figure 2.

TABLE 1. HISTOLOGICAL CHARACTERS OF CERTAIN SPECIES OF PRUNUS ROOTSTOCKS

	Prunus				
	Armeniaca	Davidiana	Persica	Amygdalus	Cerasifera
Vessels single or grouped.....	Grouped	Grouped	Single	Single	Single
Wood parenchyma present or absent.....	Very small amount present	Absent	Absent	Absent	Usually present in considerable amounts
Width of rays in old roots in number of cells.....	5-7	5-7	5-8	6-9	3-5
Cross-sectional shape of medullary ray cells.....	Barrel-shaped	Square	Square	Square	Rectangular, elongated radially
Presence or absence of fan-shaped ray extensions in cortex.....	Fan; starts to form midway between cambium and bark surface	No Fan	Fan; starts midway between cambium and bark surface	Fan; starts to form at cambium	Fan; starts to form at cambium

Plate 3, figure 4, shows the typical distribution of the tracheae in *Prunus Amygdalus*. It will be noted that the vessels are large and numerous in the spring wood, but diminish rapidly in number and size as the fall wood is approached. This is strikingly different from the distribution of vessels in *Prunus Persica*.

*Prunus cerasifera*, Myrobalan plum

This root possesses no gross morphological characters that suffice for positive identification. There are, however, histological features that are of significance for this purpose. In all the previously described roots, wood parenchyma is practically lacking, the medullary ray cells are nearly square in transverse section, and the rays are from 5-9 cells in width. In *Prunus cerasifera* wood parenchyma cells are usually present in rather large numbers, the radial width of the medullary ray cells is from two to five times that of the tangential width and the rays rarely exceed five cells in width. These characters are clearly shown in plate 4, figure 1.

GROUP II. CHERRY SPECIES, *Prunus avium* AND *Prunus Mahaleb*

*Prunus Mahaleb* makes a relatively slow growth, hence it is sometimes outgrown by the scion, which causes a constriction at the point of union. This does not occur with *Prunus avium* (Mazzard). The difference in the rate of growth between the stock and scion does not usually make itself apparent until the trunk attains a diameter of about six inches. Many use this constriction as a means of distinguishing one root from the other. The writer, however, believes this method of identification is too indefinite, although the conclusions drawn may be correct in many cases. As the effect of constriction is not manifested in the gross morphology of a portion of a root, it is evident that other means must be used for identification.

From a morphological point of view, these two species of roots are indistinguishable. Color and lenticels are practically the same in both roots. As a rule, young roots are light yellow, while the older ones appear darker in color.

As mentioned in the key, the cherry species are differentiated from the other species of *Prunus* roots by reason of the fact that calcium

oxalate crystals are present within the collenchyma cells, and also by the fact that the medullary ray extensions within the cortex usually assume a sinuous course.

Plate 4, figure 2, shows the characteristic small lumina of the wood fibres of *Prunus Mahaleb*. The tangential diameter of the cavity is generally less than the thickness of the wall. The cells themselves are small and compact in their arrangement.

In *Prunus avium* the lumina of the wood fibres are relatively large. Instead of being less in diameter than the thickness of the wood fibre wall, the cavity is generally two or three times as wide, as is seen in plate 4, figure 3. The radial diameter of the fibres of the two roots also differs. In roots two or more years old, the average size of the cells measured was as follows:

Species	Average radial diameter of wood fibres
<i>Prunus avium</i> .....	0.021 mm.
<i>Prunus Mahaleb</i> .....	0.012 mm.

One of the outstanding differences between *Prunus Mahaleb* and *Prunus avium* is the taste of the cortex. The cortex of the latter is very bitter and astringent while that of the former is not. The bitterness of the cortex of *Prunus avium* is due to the large amount of phloridzin present.\* Taste of cortex is probably the best criterion to use for the separation of the two species of cherries under discussion.

The distribution of the vessels as seen in cross-section is, as a rule, distinctly different in these species. In *Prunus avium* they are quite large and scattered rather uniformly throughout the entire year's wood growth. The vessels are generally elongated radially and attain a maximum diameter of approximately 0.12 mm. In *Prunus Mahaleb* the vessels are not uniformly scattered throughout the entire year's wood growth but are usually restricted to the wood developed early in each year. The average radial diameter of the two species differ, as is shown by the following measurements:

Species	Average radial diameter of tracheae
<i>Prunus avium</i> .....	0.110 mm.
<i>Prunus Mahaleb</i> .....	0.084 mm.

\* Wehmer<sup>11</sup> in his "Die Pflanzenstoff" gives phloridzin as a constituent of the bark of *Prunus avium*, but not of *Prunus Mahaleb*.



Plate 4, figure 4, shows the characteristic unevenness of the cork cells of *Prunus Mahaleb*. *Prunus avium* does not exhibit this irregularity in size of cork cells.

### GROUP III. PYRUS SPECIES, INCLUDING THE QUINCE

<i>Pyrus Malus</i> Linn.....	Apple
<i>Cydonia oblonga</i> , var. Angers, Mill.....	Angers quince
<i>Cydonia oblonga</i> , var. Orange, Mill.....	Orange quince
<i>Pyrus serotina</i> Rehd.....	Japanese pear
<i>Pyrus communis</i> Linn.....	French pear
<i>Pyrus ussuriensis</i> Maxim.....	Ussuriensis pear
<i>Pyrus Calleryana</i> Decne.....	Calleryana pear

Although it was found possible to determine distinguishing characters for roots of the *Prunus* species, great difficulty was encountered in doing so for the species of *Pyrus*. Ecological factors seem to have a greater influence on the roots belonging to the latter genus than on those of the former. For instance, the wood parenchyma cells in pear roots from Oregon were found in practically all cases to be larger in cross-section than the tracheae, while the reverse condition existed in roots from the Sacramento Valley of California. As far as the present investigation was carried, no prominent differences could be noted between the different species of *Pyrus* and *Cydonia* that were as definite as those established between the *Prunus* species. Differences were noted in some cases, but whether these may be used as criteria for differentiation is probably open to question. From an anatomical and morphological point of view many of the species are practically identical.

Despite the fact that roots of each species possess peculiar characteristics, such as resistance to a certain disease, it does not necessarily follow that a variation exists in the anatomy of the root. The conditions to which a stock is suited are undoubtedly dependent upon physiological, rather than upon morphological or anatomical characters.

The close phylogenetic relationship between the *Pyrus* species probably accounts for the difficulty in finding anatomical characters that could be used for distinguishing them. This difficulty does not occur with the *Prunus* species because they are not so closely related.

From the point of view of gross morphology all the roots in this group are identical. No prominent differences seem to exist in either color or character of lenticels.



*Pyrus Malus*, Apple

As is seen in the key, the chief differentiating feature of *Pyrus Malus* is the character of the cells that make up the cortex. Instead of being closely compacted, as in all the other species of *Pyrus*, the cells are large and fit loosely together. In transverse view the cells appear elliptical in outline with the longer axis in a tangential direction.

The tracheae, medullary rays and wood mass are practically identical with those of the pears and the quinces, showing that a close relationship exists between the various species of *Pyrus*.

*Cydonia oblonga*, Quince

The two varieties of *Cydonia oblonga* used in the investigation, namely, Angers and Orange, are indistinguishable by either a gross morphological or anatomical study (pl. 5, figs. 1 and 2). They are also practically identical with the pear roots, morphologically and anatomically. Owing to the dwarfing effect of the quince roots on the scion of the pear, it would probably be inferred that the quince cells are smaller than those of the pear. This is not the case, and, in fact, certain types of cells in the quince are larger than the corresponding cells of the pear.

There seems to be but one outstanding difference between the two genera, *Pyrus* and *Cydonia*, namely, the size of the lumina of the wood fibres. In the latter the lumen of the cell is a little wider than the thickness of the wall, while in the former it may be several times as wide.

*Pyrus communis* and *Pyrus serotina*, French and Japanese Pears

There are no macroscopic characters that can be used for differentiation between these two species. Microscopically, the roots are practically identical. The only variation that seems to be constant and that may be used for differentiation is the character of the vessels in the early growth of each year's wood. These variations are not always clear and distinct and can be found only when the sections studied are very thin.

Plate 5, figure 3, shows the characteristic radially-elongated and slightly angular-walled tracheae in the spring wood of *Pyrus serotina*.

This elongation is quite marked. Plate 5, figure 4, shows the more nearly isodiametric and distinctly angular-walled tracheae in the spring wood of *Pyrus communis*.

Another difference between the two species is the cross-sectional size of the tracheae in the spring wood of each year. Those of *Pyrus serotina* are uniformly larger, as is shown by the following measurements:

Species	Tangential diameter	Radial diameter
<i>Pyrus serotina</i>	.07 mm.	.096 mm.
<i>Pyrus communis</i>	.06 mm.	.072 mm.

The above are the only distinct differences that seem to exist between the two species, and hence probably the best to use for differentiation purposes. It often happens that both roots exhibit vessels that do not conform in shape or size to that which is typical for each root but instead appear as gradations between the two types of vessel forms.

Undoubtedly, a more positive means of differentiation would be preferred, but whether such exists is doubtful, especially in view of the innumerable variations that seem to be due to ecological factors.

#### *Pyrus ussuriensis* and *Pyrus Calleryana*, Ussuriensis and Calleryana Pears

These two species of *Pyrus* have been omitted from the key for two reasons, first, because of the indefiniteness of the differentiating features and, second, because there are but few plantings of these stocks in the state at the present time.

There seems to be an even closer relation between these two roots than between *Pyrus serotina* and *Pyrus communis*. The only differentiating feature is the number and arrangement of the tracheae. As a rule, the tracheal vessels of the Ussuriensis are very numerous and scattered rather uniformly through the entire wood (pl. 6, fig. 1). In most cases the distance from one vessel to another rarely exceeds the average radial diameter of the tracheae. In some cases this relationship does not exist, but if a root is sectioned and examined at several different points the typical appearance mentioned will be noticed. The vessels in the spring growth are slightly larger than those in the fall wood.

The anatomical features of Calleryana are practically identical with those of Ussuriensis, with one possible exception, namely, the tracheae are less numerous (pl. 6, fig. 2). In many cases the vessels are very few in number. As a rule, the spring growth does not possess more tracheae than the remainder of the wood, the annual ring being set off by a single row of cells, instead of a deep layer as in Ussuriensis.

The cross-sectional dimensions of the vessels in the two roots vary slightly. The average diameters obtained from numerous measurements are:

Species	Radial diameter	Tangential diameter
Pyrus ussuriensis	.070 mm.	.056 mm.
Pyrus Calleryana	.060 mm.	.040 mm.

The above figures show that the difference which exists between the average sizes of the tracheae of the two roots is of sufficient significance to be of value in identification. However, the visual impression of the difference in size is a better guide than actual measurements, owing to the considerable variation between cells of the same root.

It thus appears that the differentiating features between *Pyrus ussuriensis* and *Pyrus Calleryana* and between *Pyrus communis* and *Pyrus serotina* are rather indefinite. It is doubtful whether these differences are sufficiently marked to allow of positive identification unless a large number of sections are studied.

SUMMARY AND CONCLUSIONS

Ecological variations cause marked differences in gross morphological and certain anatomical features of fruit tree rootstocks, this influence being greater on the roots of *Pyrus* than on those of *Prunus*.

Roots of species of *Pyrus* may be separated from roots of *Prunus* by a histological study of the following: Medullary rays, shape of tracheae in cross-section and nature of heart and sap wood.

Except with *Prunus Armeniaca*, color should not be used when trying to identify deciduous fruit tree rootstocks. *Prunus Armeniaca* may be distinguished by the beet-red color of its roots possessed by none of the others.

*Prunus Amygdalus* may be distinguished by the character of its lenticels.

*Prunus Persica* and *Prunus Davidiana* are identical from a gross morphological point of view, but are strikingly different in certain anatomical features. The tracheae in the former are distributed singly while in the latter they are in groups.

*Prunus cerasifera* may be distinguished with certainty only by a histological study of its medullary ray cells and heart and sap wood.

From a gross morphological point of view, *Prunus Mahaleb* and *Prunus avium* are indistinguishable. The bitter and astringent taste of *Prunus avium* is usually sufficient to distinguish it from *Prunus Mahaleb*. Histologically, the roots are strikingly different from each other.

The differences observed between the various species of *Pyrus* are rather indefinite in some cases, which renders them of little value for identification.

The chief differentiating feature of *Pyrus Malus* is the large, loosely fitting cells that make up the cortex.

Although the quinces belong to a different genus (*Cydonia*), their roots are practically identical with those of the genus *Pyrus*, morphologically and histologically. The only differentiating feature is the size of the lumina of the wood fibres; in *Cydonia* the lumen is slightly wider than the thickness of the wall, while in *Pyrus* it may be several times as wide.

The best, and probably the only means of distinguishing *Pyrus serotina* and *Pyrus communis* from one another is by a study of the tracheae, in transverse section, in the spring wood of each year. In the former the vessels are elongated radially and only slightly angular-walled, while in the latter they are more nearly isodiametric and distinctly angular-walled.

A closer histological relationship exists between *Pyrus ussuriensis* and *Pyrus Calleryana* than between *Pyrus serotina* and *Pyrus communis*. The only differentiating feature is in the number and arrangement of the tracheae.

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## PLATE 1

Fig. 1. Transverse section showing distinguishing features of *Pyrus* roots. Note narrow rays (one or two cells in width), angularity of tracheal walls, and numerous wood parenchyma cells.

Fig. 2. Transverse section showing distinguishing features of *Prunus* roots. Note wide ray, smoothness of tracheal walls, and lack of wood parenchyma cells.

Fig. 3. Transverse section of *Prunus Armeniaca* showing barrel-shaped cells in medullary rays. Note grouped tracheal vessels.

Fig. 4. Transverse section of *Prunus Armeniaca* showing fan-shaped ray extension in cortex. Note that fan does not start to form until the ray is well into the cortex.



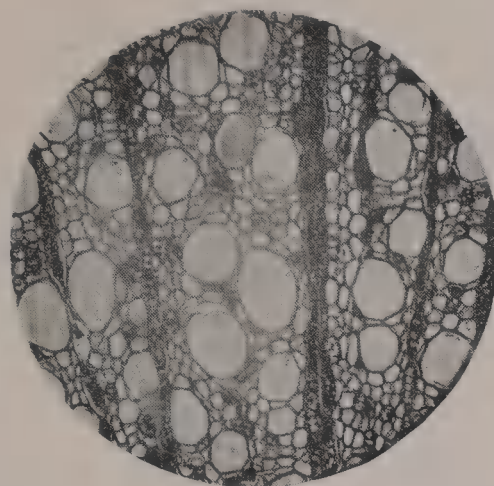


Fig. 1

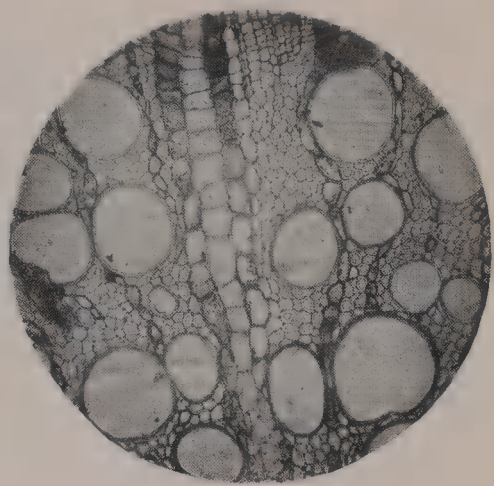


Fig. 2

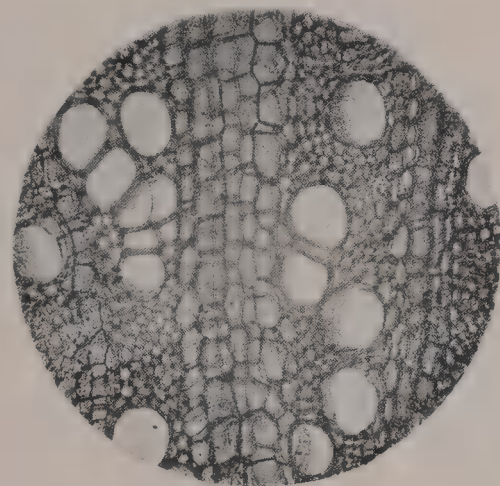


Fig. 3

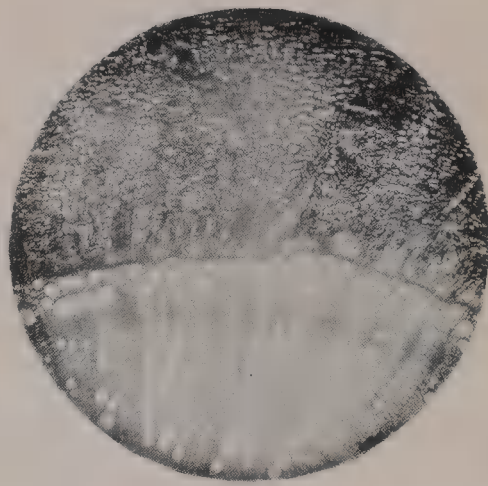


Fig. 4





## PLATE 2

Fig. 1. Showing bark of *Prunus Amygdalus* (left) and *Prunus Persica* (right). Note roughness of bark of *Prunus Amygdalus* and smooth bark of *Prunus Persica*. Observe variations in lenticel characters: distinct slit in those of *Prunus Amygdalus* and indefiniteness of same in *Prunus Persica*; variation in longitudinal height; smooth lenticels of *Prunus Amygdalus* and rough lenticels of *Prunus Persica*. Note lateral and longitudinal cracks in bark of *Prunus Persica* and lack of same in bark of *Prunus Amygdalus*.

Fig. 2. Showing variation in thickness of cortex in roots of *Prunus Persica* (left) and *Prunus Amygdalus* (right).

Fig. 3. Transverse section of *Prunus Davidiana* showing numerous groups of tracheae. Note lack of wood parenchyma.



Fig. 1

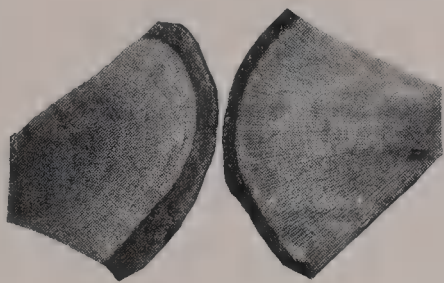


Fig. 2

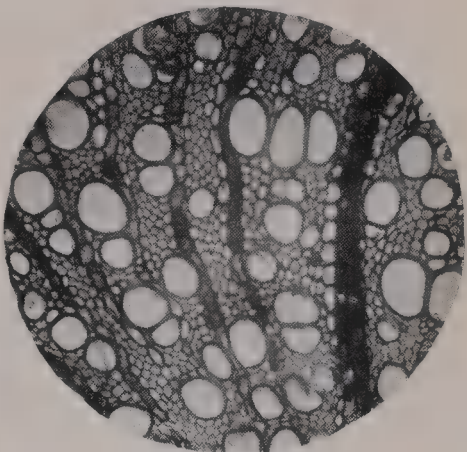


Fig. 3







### PLATE 3

Fig. 1. Transverse section of root of *Prunus Persica* showing even distribution of tracheal vessels. Note gradual diminution in size of vessels as the fall wood is approached and also the roundness of the first vessels formed in each annual ring. Compare with figure 4.

Fig. 2. Transverse section of root of *Prunus Persica* showing the fan-shaped medullary ray extension in the cortex. Note that the fan does not start to form until the ray reaches the center of the cortex.

Fig. 3. Transverse section of *Prunus Davidiana* showing lack of fan in cortex.

Fig. 4. Transverse section of root of *Prunus Amygdalus* showing uneven distribution and size of tracheal vessels. Note elongated nature of first tracheae formed in each annual ring and abrupt change of radial diameter of vessels as the fall wood is approached. Compare with figure 1.

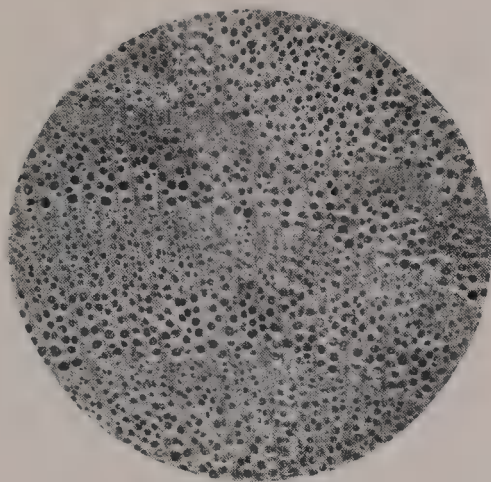


Fig. 1

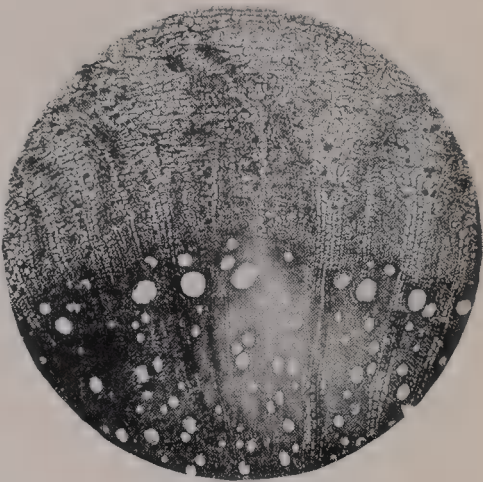


Fig. 2

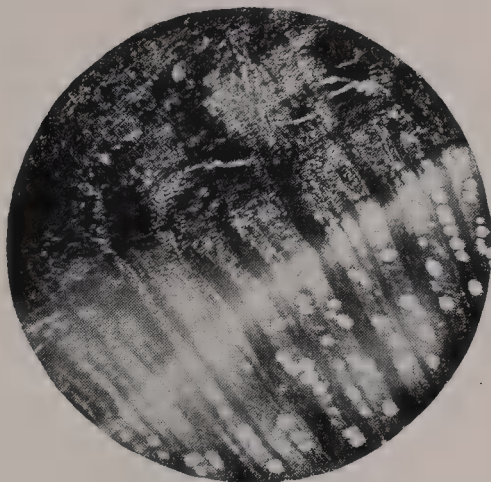


Fig. 3

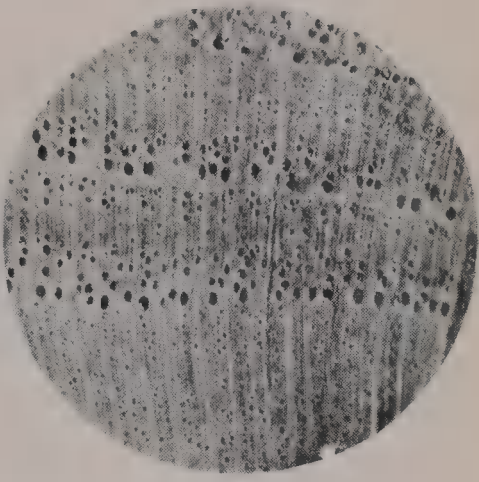


Fig. 4





#### PLATE 4

Fig. 1. Transverse section of *Prunus cerasifera* showing numerous wood parenchyma cells and elongated nature of medullary ray cells.

Fig. 2. Transverse section of *Prunus Mahaleb* showing small lumina of wood fibres. Compare with lumina of same cells of *Prunus avium*, figure 3. Note thickness of wood fibre wall.

Fig. 3. Transverse section of *Prunus avium* showing large lumina of wood fibres. Note relatively thin walls of wood fibres.

Fig. 4. Transverse section of *Prunus Mahaleb* root showing irregularity in size of cork cells and calcium oxalate crystals within collenchyma cells.



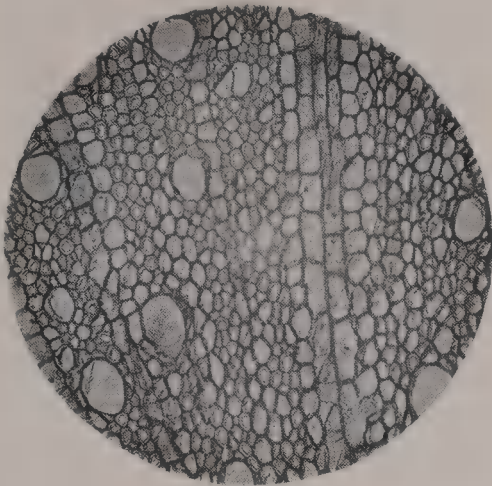


Fig. 1

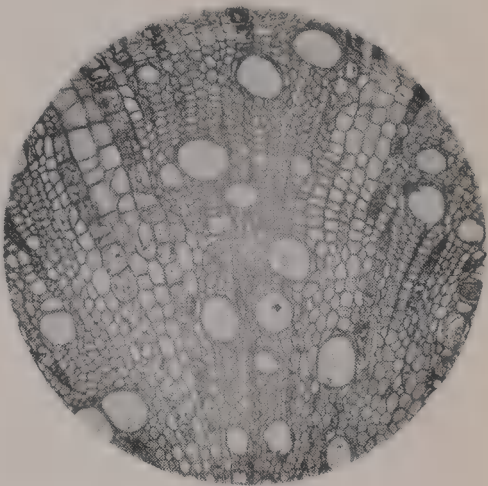


Fig. 2

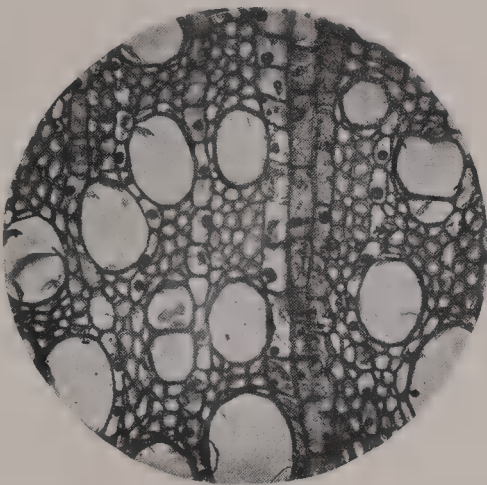


Fig. 3

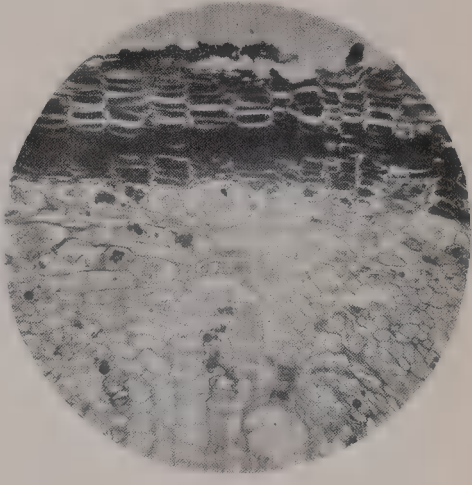


Fig. 4





## PLATE 5

Fig. 1. Transverse section of Angers quince root showing relative size and distribution of tracheal vessels.

Fig. 2. Transverse section of Orange quince root showing same as figure 1. Note degree of similarity between figures 1 and 2 and also compare with *Pyrus Calleryana*, plate 6, figure 2.

Fig. 3. Transverse section through annual ring of root of *Pyrus serotina* showing elongated and slightly angular-walled tracheae.

Fig. 4. Transverse section through annual ring of root of *Pyrus communis* showing isodiametric and angular-walled tracheae.

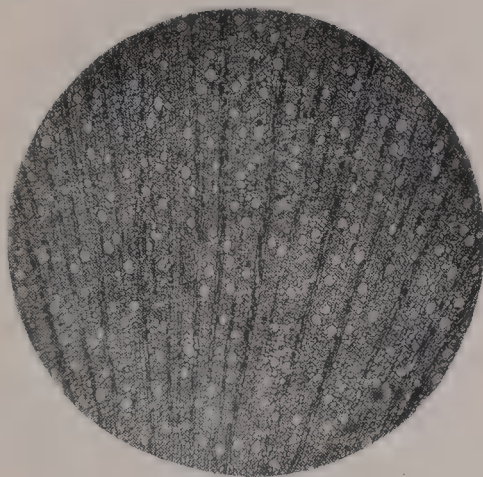


Fig. 1

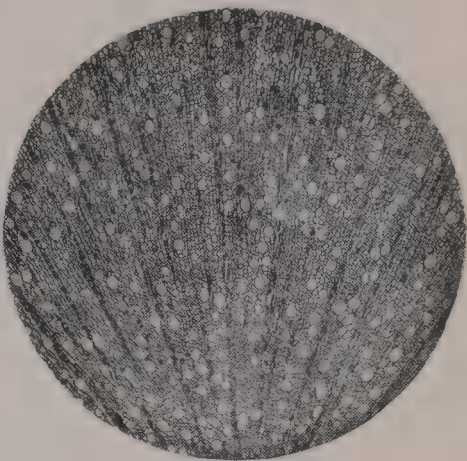


Fig. 2

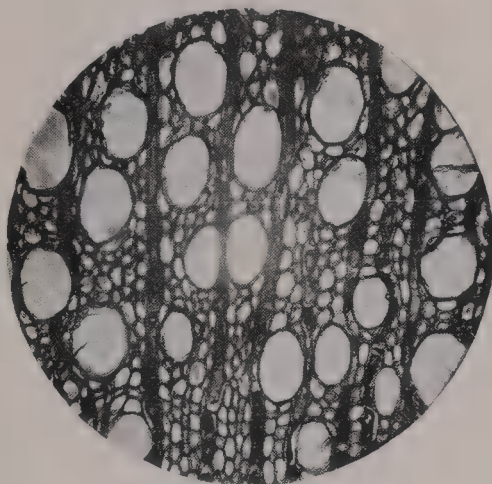


Fig. 3

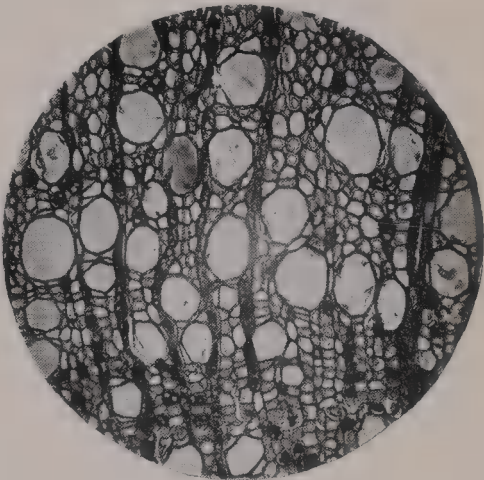


Fig. 4







PLATE 6

Fig. 1. Transverse section of *Pyrus ussuriensis* showing numerous and uniformly scattered tracheae.

Fig. 2. Transverse section of *Pyrus Calleryana* showing relative size and manner of distribution of tracheae.

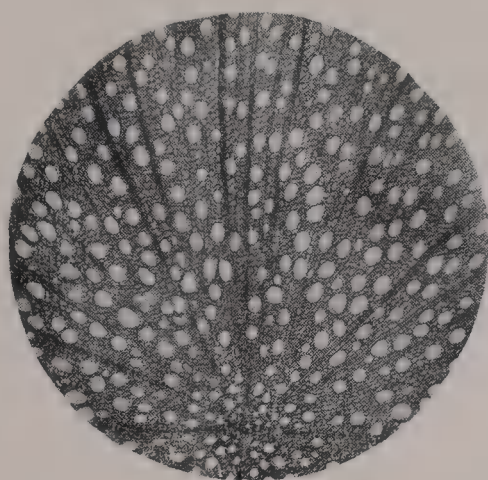


Fig. 1

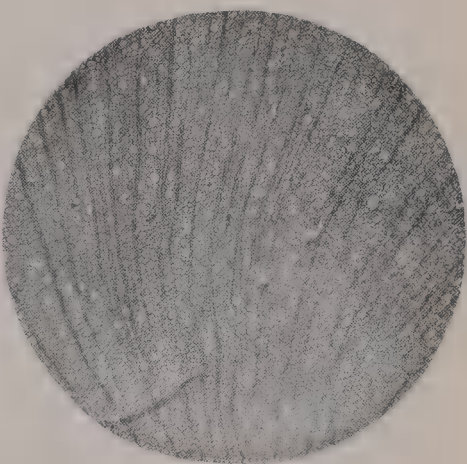


Fig. 2



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JUNE, 1923

TECHNICAL PAPER No. 7

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A STUDY OF THE DARKENING OF  
APPLE TISSUE

BY

E. L. OVERHOLSER AND W. V. CRUESS

UNIVERSITY OF CALIFORNIA PRESS  
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A STUDY OF THE DARKENING OF  
APPLE TISSUE

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E. L. OVERHOLSER AND W. V. CRUESS

Contribution from the Divisions of Pomology and Viticulture and Fruit Products. College of Agriculture and Agriculture Experiment Station, University of California, Berkeley, California.

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Apples to be dried are usually treated with the fumes of burning sulfur, the principal purpose being to prevent the darkening of the fruit. This treatment is used so extensively that a clear understanding of the process is desirable, particularly in view of the fact that objections have been raised to such use of sulfurous acid. It was anticipated that a more complete knowledge of the action of this compound and of other factors that influence the color of fruit, might suggest a method by which the use of sulfurous acid could be restricted or avoided. The investigation reported in this paper was made to obtain the required data.

THE OXIDASE SYSTEM

It is generally accepted that oxidases are concerned in the oxidation of a large number of substances in plants. According to Kastle and Loevenhart,<sup>13</sup> laccase, or as referred to by Browne,<sup>6</sup> a "ferment," is responsible for the color changes which occur in cut fruits such as apples and pears. The brown color formed is believed to be caused by the oxidation of tannin. The browning of cider after pressing is similarly explained.

At one time a tissue was thought to contain an *oxidase* when it possessed power to catalyse oxidations of natural or of added chromogens. Those tissues requiring the addition of a peroxide, such as

hydrogen peroxide, to effect oxidation, were thought to contain a *peroxidase*. The view is gaining ground, however (Bach and Chodat,<sup>3</sup> Moore and Whitley,<sup>17</sup> and others) that oxidases are not entities but in reality mixtures of a peroxidase and an unstable organic peroxide.

Ewart<sup>9</sup> states that there is no justification for the use of such terms as peroxidase, catalase, enoxidase, and tyrosinase to indicate specific substances, ferments, or groups of ferments. In another paper<sup>10</sup> he states that it is permissible, however, to use such terms as catalase action or peroxidase action. He further states that comparison with metallic oxidases shows that there is no reason for assuming the existence of specifically distinct plant oxidases.

Some of the experiments reported in this paper indicate the presence in certain fruits of substances comparable to organic peroxides and peroxidases.

*Definition of Terms.*—The terms occurring in this report have been employed with meanings as follows:

1. The *oxidizing system* is considered to consist of a peroxidase and an organic peroxide and is designated by the term *oxidase*.

2. The *peroxidase* is considered to be that part of the oxidizing system which has the power to cause the rapid transfer of the oxygen derived from the organic peroxide—normally occurring in most fruits, or derived from hydrogen peroxide—to the compounds which undergo oxidation, and behaving as an accelerator of this oxidation process.

3. The *organic peroxide* is considered to be that portion of the oxidizing system which, in the presence of the peroxidase, liberates oxygen in an active state.

4. The *chromogen* is considered to be a substance of tannoid nature found in apples, which upon being oxidized, gives rise to a brown pigment.

#### NATURE OF DARKENING

To obtain a better understanding of the problem, a preliminary study was made of the nature of the darkening process. It was desired to determine the relative importance of the factors concerned in the darkening of cut or otherwise injured apple tissue. The factors especially studied were the peroxidase and the organic peroxide, although the chromogen was also included.

In order to find suitable indicators for the presence of peroxidase and organic peroxide in fruits, several reagents were tested.

*Test for Peroxidase.*—The peroxidase indicators were tested with both juice and scarified apple tissue. Fresh juice from Yellow Newtown apples was obtained by grinding the fruit and pressing through several thicknesses of cheese cloth. Five cubic centimeter portions of the juice were placed in test tubes. To duplicate samples were added a few drops of the following solutions: (a) benzidine (2% in 50% alcohol); (b) dilute tincture of guaiac; (c) guaiacol (2% in 95% alcohol); (d) alpha-naphthol (1% in 50% alcohol); (e) phenol (5% in water). Two untreated tubes of juice were reserved as checks.

Fresh apples were also cut into slices five or six millimeters thick and the upper surfaces of the slices were scarified and treated with the reagents noted above.

Observations were made upon the fresh juice after five minutes, thirty minutes, and thirteen hours, and upon the scarified tissues after twenty minutes, forty-five minutes, and three hours. The data are summarized as follows:

1. Benzidine, tincture of guaiac, guaiacol, and alpha-naphthol gave color reactions of oxidation with both fresh juice and fresh apple tissue.

2. Onslow<sup>20</sup> states that alpha-naphthol, and benzidine are oxidized by oxidases only with the assistance of hydrogen peroxide. We found, however, that the oxidase system of Yellow Newtown apples was sufficient to oxidize each reagent without the addition of hydrogen peroxide.

3. Benzidine was the most satisfactory indicator for the oxidizing system. It rapidly gave a pronounced blue or purple color. The statement is frequently made that auto-oxidation of benzidine may take place in air, if the observation interval is too prolonged. We found, however, no evidence of auto-oxidation of benzidine upon boiled apple tissue, even after seventy-two hours.

4. Tincture of guaiac was next in desirability, but it was erratic in behavior and the blue coloration was masked by the murky brown of the solution.

5. Guaiacol gave a marked brown coloration which, however, was difficult to distinguish from the darkening of untreated checks. Furthermore, the color was slow in developing.

6. Alpha-naphthol gave an appreciable violet color, but this developed so slowly that its use was impracticable.

7. Phenol developed coloration very slowly and the end color was difficult to distinguish from the normal browning of untreated juice.

*Tests for Organic Peroxides.*—Two indicators were used in testing for the presence of organic peroxides: (a) chromic acid, and (b) potassium, iodide and starch. Acetic acid was used in test (b) instead of the ferrous sulfate usually recommended, because the latter reacted with the tannin of the juice, giving a greenish color which obscured the peroxide reaction.

(a) Hydrogen peroxide and an acidified solution of potassium dichromate gives rise to an unstable combination of chromic acid with hydrogen peroxide. On mixing the solution with ether this compound can be extracted from the aqueous solution and imparts to the ether a characteristic blue color. Fresh juice of Newtown apples placed in a dilute solution of chromic acid gave a deep blue color in the ether extract, which was considered as a positive test for peroxide.

(b) When hydrogen peroxide is added to a solution of potassium iodide, iodine is liberated and causes starch to become blue. This test was used upon apple tissue as follows: The apple was cut into thick slices, one exposed surface of the tissue was scarified, and dilute solutions of potassium iodide and starch were added. With fresh apple tissue marked blue coloration, indicating a positive test for peroxides, became apparent in from ten to fifteen minutes.

The first test (a) proved satisfactory for apple juice, while the second test (b) was preferable for apple tissue.

#### A STUDY OF THE COMPONENTS OF THE OXIDIZING SYSTEM

An effort was made to isolate the oxidizing enzymes involved in the browning process. Expressed apple juice was treated with two parts of 95% ethyl alcohol. After continued mixing the precipitate was removed by filtration and re-dissolved in water. This treatment was employed with juices of Newtown apples obtained under the following conditions: (a) juice permitted to brown after extraction; (b) juice extracted from fresh Newtown apples at a temperature of 0° C. and treated with alcohol before browning occurred; (c) juice from sulfured apples and hence not brown in color, and (d) juice from boiled, sliced apples which had not browned.

(1) *Separation of Oxidizing System from Chromogen.*—The possible separation of the oxidizing enzyme, organic peroxide, and tannin

by the above means, was determined by testing both the alcoholic filtrate and the re-dissolved residue or enzyme extract for the presence of these substances. The data obtained are tabulated in table 1.

TABLE 1

TESTS FOR PEROXIDASE, ORGANIC PEROXIDE AND TANNIN, IN PORTIONS OF APPLE JUICE, TREATED TO SEPARATE THESE CONSTITUENTS

Portion used	Tests		Organic peroxide test, KI—starch—acetic acid	Tannin test with dilute $\text{FeCl}_3$
	for oxidase Benzidine only	for peroxidase Benzidine and $\text{H}_2\text{O}_2$		
Browned juice .....	+	++	+	+
Enzyme extract from browned juice..	+	++	+	—
Filtrate from browned juice.....	—	—	—	+
Unbrowned juice.....	++	++	++	+
Enzyme extract from unbrowned juice.....	++	++	++	—
Filtrate from unbrowned juice.....	—	—	—	+
Sulfured juice.....	—	+	—	+
Enzyme extract from sulfured juice ..	—	+	—	—
Filtrate from sulfured juice .....	—	—	—	+
Boiled juice.....	—	—	—	+
Enzyme extract from boiled juice.....	—	—	—	—
Filtrate from boiled juice.....	—	—	—	+

Note:—Positive reaction is indicated by +; strong positive by ++; and negative by —.

The data indicate there is an oxidizing system rather than a single substance involved in the browning of the Newtown apple juice. For example, sulfuring of the apple juice destroys the organic peroxide and incidentally the oxidizing system, but apparently does not affect the peroxidase or the oxidizable tannoid substance. They further indicate that the peroxidase can be separated from apple juice and that the organic peroxide is carried down with the peroxidase in the precipitate. In the browned juice and the enzyme extract from the same, the test for organic peroxide was not so marked as in the case of the unbrowned, supposedly because the browning had utilized some of the original organic peroxide.

The tannin, or the substance which becomes brown upon oxidation, is soluble in alcohol and hence is separated from the oxidizing system when the latter is precipitated with alcohol. Otherwise, the reactions of the enzyme extract as determined in table 1 are identical with those of the original juice. By "enzyme extract" is meant the water solution of the precipitate resulting from addition of alcohol.



(2) *Reaction between Oxidizing System and Apple Tannin.*—The enzyme extract, containing peroxidase and organic peroxide, was added to the filtrate containing the tannin substance to determine whether the chromogen would brown. Two cubic centimeters of the enzyme extract obtained from both browned and unbrowned juices were added to ten cubic centimeters of the tannin filtrate. The data are summarized in table 2.

TABLE 2  
OXIDATION OF APPLE TANNINS BY THE ADDITION OF THE TANNIN FREE  
OXIDIZING SYSTEM

Source of tannin	Source of enzyme extract	Further treatment	Observations		
			After 5 minutes	After 12 hours	After 24 hours
Boiled juice.....	None	None	No change	No change	No change
Boiled juice.....	Browned juice	None	Browned	Browned	Browned
Boiled juice.....	Browned juice	H <sub>2</sub> O <sub>2</sub>	Browned	Browned	Browned
Boiled juice.....	None	H <sub>2</sub> O <sub>2</sub>	No change	No change	No change
Non-browned juice	None	None	No change	No change	No change
Non-browned juice	Non-browned juice	None	Browned	Browned	Browned
Non-browned juice	Non-browned juice	H <sub>2</sub> O <sub>2</sub>	Browned	Browned	Browned
Non-browned juice	None	H <sub>2</sub> O <sub>2</sub>	No change	No change	No change

The data in table 2 shows that tannin separated from the oxidizing system can be oxidized brown when the two are again placed together. This indicates that treatment with alcohol destroys neither the tannin nor the component parts of the oxidizing system.

(3) *Effect of Inorganic Catalytic Agents upon the Browning of Boiled Apple Juice.*—Slices of Newtown apples were dropped into sufficient boiling water to barely cover the mass, and boiled to destroy the peroxidase and organic peroxide. The slices were then ground in a meat chopper and the juice expressed through several thicknesses of cheese cloth. Samples of 10 c.c. each of the juice were placed in test tubes. This juice gave a positive reaction for tannin with ferric chlorid but a negative reaction for peroxide.

In order to determine whether the tannin could be browned by supplying inorganic catalytic agents and hydrogen peroxide, manganese dioxide and platinizing solution were added to samples of the juice.



As shown elsewhere, high hydrogen-ion concentration retards the browning of freshly expressed juice. Hence the juice in one lot of test tubes was nearly neutralized by  $\text{CaCO}_3$ , but not enough was added to bring about darkening due to alkalinity. The data obtained are summarized in table 3.

TABLE 3  
THE EFFECT OF INORGANIC CATALYTIC AGENTS UPON THE BROWNING OF  
BOILED JUICE

Juice	Treatment	Appearance after 30 minutes	Appearance after 3 hours
Boiled	None (check)	No browning	No browning
Boiled and neutral- ized	None (check)	No browning	Slight browning at surface
Boiled	Two drops $\text{H}_2\text{O}_2$	No browning	No browning
Boiled and neutral- ized	Two drops $\text{H}_2\text{O}_2$	No browning	Slight browning throughout
Boiled	Trace of $\text{MnO}_2$	No browning	No browning
Boiled and neutral- ized	Trace of $\text{MnO}_2$	No browning	Slight browning at surface
Boiled	Trace $\text{MnO}_2$ Plus $\text{H}_2\text{O}_2$	No browning	No browning
Boiled and neutral- ized	Trace $\text{MnO}_2$ Plus $\text{H}_2\text{O}_2$	Browning throughout	Dark browning throughout
Boiled	1 drop platinizing solution	No browning	Slight browning throughout
Boiled and neutral- ized	1 drop platinizing solution	Slight browning throughout	Marked browning throughout
Boiled	1 drop platinizing solution plus $\text{H}_2\text{O}_2$	No change	Slight browning throughout
Boiled and neutral- ized	1 drop platinizing solution plus $\text{H}_2\text{O}_2$	Slight browning throughout	Marked browning throughout

The data show that boiled juice alone did not darken, but boiled juice of reduced acidity darkened when an inorganic catalyzer and hydrogen peroxide were added.

Boiled juice of natural acidity did not brown except when platinizing solution was added. Slight browning resulted with and without the hydrogen peroxide.

The boiled, neutralized juice darkened slightly at the surface without the addition of hydrogen peroxide, probably because of the capacity of tannins to absorb oxygen in neutral or alkaline solutions.

## NATURE OF CHROMOGEN

It has been generally assumed that the brown color occurring in cut apples is due to the oxidation of tannin by laccase.<sup>6, 14, 16</sup> Natural tannins are powerful reducing agents and exhibit a marked tendency to absorb oxygen, particularly in an alkaline solution. The oxidation products are strongly colored. According to Buchner<sup>7</sup> and Hotter,<sup>12</sup> the tannin content of apples (*Pyrus Malus*, L.) may be from 0.1 to 0.3 per cent of the fresh weight, according to the variety.

Tests were conducted to substantiate the assumption that tannins are normally present in apple tissue and juices, and that these are the substances oxidized to a brown color. Newtown apples from cold storage were sliced, treated in various ways, with the exception of the fresh apple juice, and passed through a meat chopper. The boiled juice was prepared by thinly slicing fresh Newtown apples and quickly dropping them into barely enough boiling water to cover the fruit. The boiling water destroyed the peroxidase and organic peroxide before browning had resulted. Sulfured juice was obtained from whole apples previously subjected for twenty-four hours to sulfur fumes in a closed container.

The tannic and pyrogallie acids employed as checks were used in one per cent aqueous solutions; samples of 10 c.c. each were placed in test tubes and an excess of the reagents as given in table 4 was added.

In addition to the reagents listed in table 4, lactic acid was also employed. All tests with lactic acid, however, gave no color reaction.

The data in table 4 indicate that the chromogen in apple juice is of tannoid nature; its behavior resembles that of solutions of tannic and pyrogallie acids.

(1) Apple juice gave the characteristic coloration for one group of tannins upon the addition of  $\text{FeCl}_3$ . (2) Both apple juice and tannin solutions immediately darkened upon being made alkaline. When acidified at once with  $\text{HCl}$ , the original color was restored. (3) Neither boiling, sulfuring, nor sulfuring and boiling the apple juice affected the chromogen.

The tannin-like compound of apple juice apparently belongs to the group of tannins giving a greenish coloration with  $\text{FeCl}_3$ , instead of to the group which gives a blue-black coloration. Several tests were employed to identify more closely the tannin compound in the apple.

The tannins have been divided into two classes, according to their color reaction with ferric chlorid: (1) iron-bluing tannins, and (2) iron-greening tannins. It is considered that the former are pyrogallie acid compounds, or are derived from gallic acid, and that the latter are derived from protocatechuic acid or catechol. Uloth<sup>29</sup> states that catechol is formed in the "dry distillation of all tannins which give a green coloration with iron chlorid."

TABLE 4  
TEST FOR CHROMOGEN (TANNOID BODIES) WITH VARIOUS REAGENTS

Substance Tested	Reagent Employed	Immediate Reaction	Reaction after 24 hours
Fresh juice	None	Original color light brown	Dark brown
Boiled juice	None	Original color whitish	None
Boiled sulfured	None	Original color whitish	None
Sulfured juice	None	Original color whitish	None
Tannic acid	None	Originally, clear, colorless	Light brown
Pyrogallie acid	None	Originally, clear, colorless	Light brown
Fresh juice from untreated apples	FeCl <sub>3</sub>	Greyish green	Greyish green
Juice from boiled apples	FeCl <sub>3</sub>	Dark green	Dark green
Boiled juice from sulfured apples	FeCl <sub>3</sub>	Dark green	Dark green
Tannic acid	FeCl <sub>3</sub>	Deep black	Heavy black ppt.
Pyrogallie acid	FeCl <sub>3</sub>	Deep reddish brown	Blue black ppt.
Fresh juice	Made alkaline with NH <sub>4</sub> OH	Dark chocolate brown bleaches with HCl	Dark chocolate brown
Boiled apple	Made alkaline with NH <sub>4</sub> OH	Medium reddish brown bleaches with HCl	Medium reddish brown
Sulfured juice	Made alkaline with NH <sub>4</sub> OH	Medium reddish brown bleaches with HCl	Medium reddish brown
Boiled juice	Made alkaline with NH <sub>4</sub> OH	Light reddish brown bleaches with HCl	Light reddish brown
Tannic acid	Made alkaline with NH <sub>4</sub> OH	Dark reddish brown bleaches with HCl	Intense clear brown
Pyrogallie acid	Made alkaline with NH <sub>4</sub> OH	Deep reddish brown bleaches with HCl	Intense clear brown

Proctor<sup>21</sup> states that a precipitation of tannin with bromine water indicates the presence of a so-called catechol or phlobatannin.

Stiasny<sup>27</sup> discovered that when an aqueous solution of a so-called catechol tannin is treated with formaldehyde and hydrochloric acid and gently warmed, the tannin is completely precipitated. Pyrogallie acid tannins do not yield an entirely insoluble compound under the same conditions.

All natural tannins are completely precipitated by lead acetate solution, as shown by the fact that the filtrate from the precipitate does not give a reaction with ferric chlorid. Stiasny and Wilkinson<sup>28</sup> state that in the case of catechol or phlobatannins this precipitate is dissolved by dilute acetic acid, whereas with the gallotannins the lead compounds are insoluble or but partly soluble. The test is preferably made by adding 10 c.c. of 10% acetic acid to 5 c.c. of the tannin solution, then adding 5 c.c. of 10% lead acetate solution. No precipitate is produced with catechol or phlobatannins.

Böttinger<sup>5</sup> states that catechol in aqueous solution is precipitated by an ammoniacal solution of calcium chlorid.

According to Wislicenus,<sup>31</sup> catechol gives with ferric chlorid a green coloration which turns violet on addition of ammonia or sodium acetate.

Proctor<sup>21</sup> has outlined additional tests for the qualitative recognition of tannin substances. Of these the following were employed: (a) the nitrous acid reaction, (b) copper sulfate and ammonia, (c) stannous chlorid and hydrochloric acid, (d) concentrated sulfuric acid, and (e) sodium sulfate.

An aqueous solution of the tannoid substance of fresh apples was prepared according to methods recommended by Onslow.<sup>20</sup> Yellow Newtown apples were sliced very thin, and dropped into 95% alcohol. The fruit, after a few minutes standing in the alcohol, was ground finely in a food chopper and pressed. The mixture of alcohol and juice was filtered. The clear filtrate was distilled over a steam bath to remove excess alcohol. The aqueous residue was again filtered and lead acetate added to the filtrate to precipitate the tannins. The precipitate was separated from the liquid by filtration and was decomposed with dilute sulfuric acid. The lead sulfate was removed by filtration and the excess sulfuric acid in the filtrate neutralized with sodium carbonate. This solution was placed in test tubes in 10 c.c.

portions and the reactions compared with those of dilute solutions of purified tannin crystals (oak tannin), impure tannic acid (oak tannin), and pyrogallie acid as indicated in table 5.

TABLE 5

COLOR REACTIONS OF PURIFIED APPLE-TANNIN, OAK-TANNIN, AND PYROGALLIC ACID

Reagent	Reaction of Purified Apple-Tannin	Purified Gallic Acid Crystals	Catechol* Tannin	Pyrogallie Acid
1. Ferric alum	Dark green color	Deep blue color	Green color	Blue black, turning green and brown
2. Bromine water	Brown precipitate	No reaction	Precipitate	No reaction
3. Nitrous acid	Yellow color	Reddish brown color	Yellow color	Yellow color
4. Copper sulfate and ammonium hydroxide	Clear	Brownish green	Green color	
5. Sodium sulfite	Green color			
6. Stannous Chlorid in HCl	No reaction	Yellow color	No reaction	No reaction
7. Concentrated sulfuric acid	No reaction	No reaction	No reaction	No reaction
8. Deal shaving and HCl	Greenish ring at contact of two liquids	Green ring	Greenish ring	Brown color
9. Ammoniacal calcium chlorid solution	No reaction	No reaction		No reaction
10. $\text{NH}_4\text{OH}$	Yellow precipitate	Greenish precipitate	Precipitate	
11. Digestion with HCl, $\text{HNO}_3$ and $\text{H}_2\text{SO}_4$ respectively	Light brown	Reddish brown	Brown	Brown color
12. Excess NaOH	Darkens on standing	Darkens on standing		Darkens rapidly
	Reddish precipitate in all three cases	No reaction	Precipitate	
	Light brown color.	Reddish brown color.	Brown	Brown color
	Darkens on standing	Darkens on standing		Darkens rapidly
13. Lead acetate and dilute acetic acid	Precipitate dissolved	Precipitate does not dissolve	Precipitate dissolved	

\* After Proctor.



These reactions show that tannin from Yellow Newtown apples more closely resembled the catechol tannins than pyrogallie acid or gallic acid tannins. The reactions resembled closely those given by catechol tannins, according to the literature on tannins. It is probable, however, that the compound is of more complex nature than catechol, but contains a catechol grouping in the molecule. Catechol tannins are characterized by a more marked tendency than others to darken in alkaline solutions. Apple-tannin solution exhibited this same tendency. The green color with ferric salts and a precipitate with bromine water are considered specific for catechol tannins by Allen.<sup>1</sup>

(a) *Relation of Purified Apple Tannin to Chromogen.*—To determine whether the purified apple-tannin solution was identical with the substance which in normal apple juice darkens by oxidation, an aqueous solution of the oxidase precipitated from crushed browned apple tissue by alcohol, was added to the purified apple-tannin solution. This oxidase solution gave a negative test for organic peroxide, but a marked positive test for peroxidase. To another portion of the tannin solution, the oxidase solution and hydrogen peroxide were added. The oxidase solution alone caused no browning, but the oxidase solution plus hydrogen peroxide caused darkening of the solution in thirty minutes. Oxidase precipitated from fresh unbrowned apple juice by alcohol caused browning of the solution without addition of hydrogen peroxide. This latter oxidase extract, however, gave a marked test for organic peroxide. The purified tannin extract, therefore, reacted with apple oxidase in a manner similar to that of the substance in apples which browns through enzyme action.

(b) *Removal of Tannin by Decolorizing Carbon.*—Portions of fresh untreated juice, sulfured juice, boiled juice, and boiled sulfured juice, made alkaline with sodium carbonate, were treated with an excess of Noirit, a vegetable decolorizing charcoal, and filtered. All filtrates were colorless. Before the addition of Noirit, all the juices gave a positive test for tannin; but, after the treatment described, none of the filtrates gave a tannin reaction with  $\text{FeCl}_3$ .

Portions of 10 c.c. each of the various treated and untreated juices previously noted were placed in test tubes; and  $\text{H}_2\text{O}_2$  plus  $\text{NaHCO}_3$ , and  $\text{NaHCO}_3$  alone, were added to different samples. In both cases, the filtrate from the fresh juice slowly darkened. The filtrates from the other juices showed no darkening, indicating that the color base or tannin had been removed by the charcoal.



(c) *Distribution of Tannin in the Apple.*—Vinson<sup>30</sup> has employed a method of determining the distribution of tannin in fruits, which consists in exposing the whole fruit to the action of any volatile nitrate, preferably ethyl nitrate. Ethyl nitrate or free nitrous acid forms deep-yellow to blood-red colors with tannic and gallic acids and the higher phenols. Fresh apples when exposed to the fumes of nitrous ether quickly become a uniform dark-brown color throughout. The epidermis becomes a somewhat darker brown than the flesh.

Any treatment that increases the permeability of protoplasm, or results in a mixing of the protoplasmic contents of the cells of the apples, will also cause rapid browning of the tannin by oxidation catalyzed by the peroxidase. For example, exposing either entire or sliced specimens of fresh Newtown apples to the fumes of chloroform, ether, benzine, carbon tetrachlorid, or carbon bisulfide causes the tissue to darken rapidly.

Hence tests were made to prove that the color produced by ethyl nitrate or nitrous ether was due to its reaction with the tannin and not to an acceleration of the enzymatic oxidation processes. Boiled or sulfured apple tissue in the absence of volatile nitrates will not brown as do untreated apples, because the boiling and the sulfuring affect the peroxidase or the organic peroxide and prevent the catalyzed oxidizing process. However, neither the boiling nor the sulfuring affect the tannin. Hence sulfured and boiled tissue, when exposed to the action of ethyl nitrate or nitrous ether, browned even more rapidly than did fresh untreated tissue, probably because the tissue was more permeable.

The test described indicated roughly that the tannin is fairly uniformly distributed throughout the Yellow Newtown apple, but that the concentration of tannin is greater in the epidermis than in the flesh.

#### EFFECT OF VARIOUS FACTORS UPON CHROMOGEN, PEROXIDASE, AND ORGANIC PEROXIDE OF APPLES

The effect of (a) temperature, (b) various gases, (c) electrolysis, (d) neutral salts, (e) alkalies, (f) acids other than sulfurous acid, and (g) sulfurous acid were studied.

Exposure of a hydrolyzing enzyme to 60° C., for some hours, generally results in loss of hydrolyzing power, and in the development

of a power to inhibit the hydrolysis which the active enzyme normally accelerates. Bayliss<sup>4</sup> attributes this phenomenon to the formation of "zymoids," which combine with the enzymes, from which they are derived, to form an inactive compound. When heated enzymes are allowed to stand in aqueous solution at room temperatures, they may undergo spontaneous reactivation. This phenomenon has also been studied in connection with oxidizing ferments.

In one test, whole Yellow Newtown apples were stored at 55° C. for twelve hours. They were then removed, cut and the scarified surface tested for peroxidase and peroxide. The test was negative for peroxide, but positive for peroxidase. Upon the addition of  $H_2O_2$ , reactions with both benzidine and guaiacum were positive, proving that the peroxidase was not destroyed by this treatment. The positive reaction was confined to the core or carpels and fibro-vascular bundles. The apples were held for five days after removal from the incubator, but the peroxide did not reappear. Evidently it had been completely utilized during storage at 55° C. and was not afterwards re-formed.

In another series, slices of Newtown apples were placed in boiling water for five minutes, then removed, cooled, and the scarified surfaces of duplicate samples treated with 1% benzidine solution, 1% alpha-naphthol, 2% guaiacol, dilute tincture of guaiac, 5% phenol solutions, and dilute  $H_2O_2$ . Two slices were left untreated as checks. All results were negative, indicating that boiling had inactivated both the peroxide and the peroxidase.

In a third test, two-gallon portions of water were heated and maintained at the temperatures given in table 6. Thin slices of apples were immersed in the water for five-minute periods for each temperature. All tests were in duplicate. At intervals of 20 minutes and 48 hours after removal from the water, tests were made for peroxidase and peroxide.

The following conclusions were drawn from the results:

1. Tests made upon heated slices of apple indicate the presence of two bodies in the tissue which affect oxidation processes. These two bodies the authors have termed "peroxidase" and "peroxide."

2. The organic peroxide was more sensitive to heat than the peroxidase. The inactivation temperature after heating for five minutes was between 71° and 73.5° C. for the former and between 90° and 100° C. for the latter.

3. No definite reactivation or re-formation of the organic peroxide and peroxidase inactivated by heat was noted after standing for 48 hours.

*Storage in Gases and Vapors.*—Whole apples were sealed in tight containers filled with various gases and vapors.

TABLE 6  
INACTIVATION TEMPERATURES FOR PEROXIDASE AND ORGANIC PEROXIDE OF  
YELLOW NEWTOWN APPLES

Tests made 20 minutes after subjection to heat

Temperature to which subjected for 5 minutes	Benzidine. Test for oxidase; i.e. peroxidase plus peroxide	Benzidine, plus H <sub>2</sub> O <sub>2</sub> . Test for peroxidase	Starch-Iodide test for organic peroxide
Room temp. (check)	Strong positive	Strong positive	Positive
55°C.	Strong positive	Strong positive	Positive
61°C.	Weak positive	Strong positive	Weak positive
65°C.	Weak positive	Strong positive	Weak positive
69°C.	Weak positive	Strong positive	Weak positive
71°C.	Weak positive	Strong positive	Doubtful
73.5°C.	Negative	Strong positive	Negative
75°C.	Negative	Strong positive	Negative
77°C.	Negative	Strong positive	Negative
79°C.	Negative	Positive	Negative
81°C.	Negative	Positive	Negative
84°C.	Negative	Positive	Negative
90°C.	Negative	Positive	Negative
100°C.	Negative	Negative	Negative

Tests made 48 hours after subjection to heat

69°C.	Weak positive	Positive	Weak positive
71°C.	Weak positive	Positive	Weak positive
73.5°C.	Weak positive	Positive	Negative
84°C.	Negative	Positive	Negative
90°C.	Negative	Positive	Negative
100°C.	Negative	Negative	Negative

Apples were stored in carbon dioxide for several weeks, one lot at atmospheric pressure and the other under a pressure of 15 pounds per square inch. While the fruit remained in the gas there was little or no darkening, but it darkened rapidly on exposure to air after removal from the CO<sub>2</sub>. Whole fruit darkened first at the surface. Apples freshly removed from the gas gave a faint positive reaction for oxidase with benzidine, but a negative test with starch-potassium-

iodide solution. Untreated apples gave strong positive reactions with both reagents. These results indicated that the organic peroxide had diminished during storage, but had not completely disappeared.

The more rapid darkening of cut surfaces of apples removed from  $\text{CO}_2$  as contrasted with untreated fruit, perhaps resulted from an increased permeability caused by the excess  $\text{CO}_2$ . Removal from the gas diminished in the fruit the  $\text{CO}_2$  concentration which may have previously exerted an inhibiting influence on the darkening process.

TABLE 7

TESTS FOR PEROXIDASE AND ORGANIC PEROXIDE IN APPLES STORED FOR 35 DAYS IN VARIOUS GASES

Gas in which stored	Condition when removed from gas	Peroxide test with KI, starch and acetic acid	Peroxide test with ferric sulfate KI and starch	Benzidine test for oxidase system	Benzidine plus $\text{H}_2\text{O}_2$ for peroxidase	Remarks
Check untreated	Normal firm	Blues slowly	Blues slowly	Blues slowly	Blues quickly	
Nitrogen	Normal firm	Blues very quickly	Blues very quickly	Blues quickly	Blues quickly	
Hydrogen	White tissues soft	Very faint bluing	Very faint bluing	Very slow bluing	Blues quickly	Cut surface browns quickly
Oxygen	Brown through-out, soft	Negative	Negative	Negative	Negative	Dark brown color may have obscured tests
Carbon dioxide	White soft	Negative	Negative	Negative	Blues slowly	Browns quickly on exposure to air

This decrease in  $\text{CO}_2$  and the intimate mixing of the cell contents, with the availability of oxygen from the air might account for the rapid darkening of the exposed surface of fruit after removal from  $\text{CO}_2$ .

Yellow Newtown apples were also stored in oxygen, hydrogen, nitrogen, and carbon dioxide, in sealed containers under five to ten pounds pressure, for 35 days.

Upon removal from the gases, specimens were immediately cut and the scarified surfaces tested for peroxidase and organic peroxide. Table 7 gives the results of these observations.

Fruit after storage in nitrogen resembled closely that stored in air. The former, however, apparently possessed a more active peroxide than the untreated fruit.



Although light in color when first removed from the hydrogen, the apples browned rapidly on exposure to air. The fact that reactions for organic peroxide were very faint might indicate that most of this substance had been used in metabolic activities during storage. Rapid browning of the fruit and its softened condition indicated increased permeability and the mixing of the enzyme and substrate.

Fruit after one month's storage in oxygen gave no visible positive test for peroxide or peroxidase, although it is possible that the dark brown color of the fruit tissue obscured the reaction. Evidently the vigorously oxidizing atmosphere in this instance brought about extensive changes.

After storage in carbon dioxide the cut surface of the fruit gave negative tests for organic peroxide, but positive tests for peroxidase. It is possible that the organic peroxide was utilized for respiration in the absence of oxygen during storage. Darkening of the tissue, however, took place rapidly on standing, in spite of the apparent absence of organic peroxide. This may be explained, as in the case of fruit stored in hydrogen, upon the hypothesis that the oxygen of the air, as a result of the increased permeability of the tissue, caused the darkening.

The difference in behavior of fruit in carbon dioxide and in nitrogen, respectively, is interesting. In the latter instance there appeared to be a cessation of metabolic activity, but no evidence of deleterious effects; whereas in the carbon dioxide the changes that occurred were marked and undesirable. Carbon dioxide is a by-product of the metabolism of fruits and apparently has a toxic action which resembles in its effect that of various other metabolic by-products upon the living organism which produces them.

The authors believe that the use of nitrogen for the storage of fruits has commercial possibilities, and we expect to conduct further experiments to determine its suitability for this purpose.

After standing at room temperature for a week in the open air, each of the samples was crushed, pressed, and the reaction of the juice was compared with that of juice from untreated apples. Table 8 gives the results of these comparisons.

The organic peroxide apparently disappeared from the juice of fruit stored in oxygen and carbon dioxide gases, but remained active in fruit stored in nitrogen. Breaking down and browning of the fruit,

after it had been stored in oxygen, hydrogen, or carbon dioxide, was accompanied by disappearance or transformation of most of the tannin, as indicated by the ferric chlorid test. The exact relation between the disappearance of the organic peroxide and the decrease in tannin is not clear. It is possible, however, that the decrease of tannin is due to its oxidation in plant tissues; and, on exposure to the air or oxygen, the tannins are further changed by oxidations and condensations to a dark red insoluble substance called phlobaphene. Furthermore, it is conceivable that the oxidation processes

TABLE 8  
REACTION OF JUICES FROM APPLES STORED IN VARIOUS GASES

Juice	Peroxide test with KI-starch solution	Oxidase test with benzidine	Peroxidase test with benzidine plus H <sub>2</sub> O <sub>2</sub>	Color reaction after addition of FeCl <sub>3</sub>	Color reaction with excess NH <sub>4</sub> OH
Untreated	Positive	Positive	Strong positive	Marked green	Dark brown
From fruit in hydrogen	Negative	Negative	Strong positive	Very faint green	Slightly darkened
From fruit in nitrogen	Strong positive	Strong positive	Strong positive	Marked green	Dark brown
From fruit in carbon dioxide	Negative	Negative	Moderate positive	Very faint green	Slightly darkened
From fruit in oxygen	Negative	Negative	Moderate positive	Very faint green	Slightly darkened

completely utilized the organic peroxide normally present, and the evidence indicates that the peroxide, if consumed, is not reconstructed.

That tannin and "organic peroxide" are not identical is indicated by the fact that the filtrate from fresh juice treated with 95% alcohol in excess gave a negative test for peroxide and a positive test for tannin; and the residue on the filter paper gave a positive test both for peroxide and peroxidase, but a negative test for tannin. These reactions are discussed in greater detail on page 5 of this article.

In a further study of the effects of gases and vapors, whole and sliced Yellow Newtown apples were placed in cans and sealed in the vapors indicated in table 8. The vapors were obtained by saturating small pieces of cotton with the reagents and placing them in small beakers in the cans beside the fruit. After 98 hours (four days), the cans were opened and the contents examined with the results shown in table 9.



These results indicate that these vapors caused the disappearance of organic peroxide in the sliced and in the whole fruit. The vapors may have acted on the organic peroxide, although it is more probable that they increased the permeability and caused the mixing of the cell contents, which resulted in the complete utilization of the organic peroxide; the darkened condition of the fruit tissues supports this

TABLE 9

EFFECT OF VARIOUS VAPORS ON THE COLOR AND ENZYME REACTIONS OF  
YELLOW NEWTOWN APPLES

Vapor	Appearance of whole fruit	Appearance of slices	Test for peroxidase with benzidine and $H_2O_2$	Test for organic peroxide with (1) Benzidine and (2) with starch—Pot. Iodide Sol.
Carbon bisulfid	Moderate browning throughout	Moderate browning throughout	Positive (instantaneous)	Negative
Formaldehyde	Most of skin normal green 50% tissue slightly brown	Moderate browning throughout	Positive (instantaneous)	Negative
Carbon Tetra-chlorid	Moderate browning throughout	Moderate browning throughout	Positive (instantaneous)	Negative
Benzine	Moderate browning throughout	Moderate browning throughout	Positive (instantaneous)	Negative
Ether	Marked browning throughout	Marked browning throughout	Positive (instantaneous)	Negative
Gasoline	Normal green	Marked browning throughout	Positive (instantaneous)	Negative
Ethyl Alcohol Alcohol 95%	Normal green with only about 5% of surface slightly browned	Marked browning throughout	Positive (instantaneous)	Negative

hypothesis. According to Zerban<sup>33</sup> the peroxide portion of the oxidase complex of cane juice is rapidly utilized in the oxidation of the polyphenols of the juice, whereas the peroxidase is much more stable and is still found in the juice after a negative test for organic peroxide is obtained. The tests substantiated our conception of two bodies—a “peroxidase” and an “organic peroxide” in the oxidizing system of apple tissue.

Onslow<sup>19</sup> believes, however, that in most plants which brown on injury the peroxidase is associated with an aromatic compound containing a catechol grouping; the association of the two substances gives rise to peroxides and a system which will then turn guaiacum blue. She found that plants which do not brown on injury do not contain a compound with the catechol grouping and that their enzymes do not catalyse the oxidation of substances with such a grouping. She found, further, that the peroxidase of plants which give an oxidase reaction and brown on injury appears to be specific in its action on the catechol grouping and does not oxidize other tannins. Our data indicate that the tannin, organic peroxide, and peroxidase are separate and distinct.

Five sound Yellow Newtown apples and one sample previously browned by benzine vapors were sealed in hydrogen in quart fruit jars. Jars containing four of the sound specimens were frozen at a temperature of  $-18^{\circ}$  to  $-13^{\circ}$  C. The other two samples were left at room temperature,  $14^{\circ}$  to  $18^{\circ}$  C. After several weeks the samples in cold storage were removed and allowed to thaw. After thawing and standing three days in hydrogen gas, these apples showed no signs of browning. Fruit frozen in air, however, darkened very rapidly on thawing. One specimen, which was removed from the hydrogen gas and allowed to thaw and stand in air, darkened slowly, showing slight surface browning after three days' exposure. The flesh inside the apple remained white. The cut surface of fruit that had been frozen and thawed in air darkened rapidly; that of the fruit frozen in hydrogen and thawed in air darkened slowly.

Fruit previously browned by exposure to benzine vapors and stored in hydrogen was not bleached by this gas. Apples stored in hydrogen at room temperature remained normal in color for several weeks.

Tests for organic peroxide and peroxidase in sound fruit stored in hydrogen were positive. Fruit subjected to benzine vapors before storage in hydrogen gave a positive reaction for peroxidase, but negative for organic peroxide.

Apparently hydrogen reduced the tendency of apple tissue to brown, but this inhibitory effect did not result because of failure of the gas to increase the permeability of the cells. This conclusion is substantiated because fruit frozen in hydrogen darkened slowly upon removal to air, whereas fruit frozen and thawed in air darkened

rapidly, although the tissues were badly "broken down," permitting the cell contents to become intimately mixed and favoring rapid reaction in both cases.

The hydrogen gas may have retarded darkening of the apple tissue: (1) by combination with some of the oxygen of the organic peroxide to form  $H_2O$ , or by exclusion of oxygen, thus rendering less oxygen available for the normal browning processes; or (2) by increasing the hydrogen-ion concentration and thereby reducing the activity of the peroxidase, though we have no evidence to this effect.

*Neutral Salts.*—Rose, Kraybill and Rose<sup>25</sup> found that the activity of purified oxidase from apple bark was greatly reduced by N/10 solutions of KCl, NaCl, LiCl, HCl,  $MgCl_2$ ,  $MnCl_2$ , or  $FeCl_3$ , but that the activity of the oxidase was slightly increased by N/10 solutions of the corresponding sulfates. They found that nitrates of K, Na, and Mg had no effect on the oxidase and that those of Ca, Ba, Mn, and Fe slightly reduced its activity. Tartrates, oxalates, citrates, acetates, and carbonates increased the rate of oxidation. Concentrations of KCl below 0.02/N exerted no effect. The authors did not state the cause of the inhibitory effort of the chlorids.

In the commercial drying and canning of apples it has been customary to keep the peeled and cut fruit in dilute salt solution.<sup>6</sup> Experiments were conducted by the authors to ascertain whether the chlorids affect the peroxidase, the organic peroxide, or the coloring matter.

Slices of Yellow Newtown apples were immersed for three days in 5% solutions of NaCl, cane sugar,  $NaNO_3$ ,  $NaNO_2$ ,  $Na_2CO_3$ , HCl,  $Na_2SO_3$ , and in tap water. The samples in  $NaNO_2$ ,  $NaNO_3$ ,  $Na_2CO_3$ , and tap water became brown in color. Those in NaCl, HCl,  $Na_2SO_3$ , and sugar were white at the end of 72 hours. The samples in  $NaNO_2$  and  $Na_2CO_3$  became alkaline in reaction. This accounts largely for the darkening in these solutions, although it was found in subsequent tests that nitrites darken apple tissue by reaction with the apple tannin. The  $Na_2SO_3$  gave  $H_2SO_3$ , which prevented darkening in the manner described elsewhere in this paper. The fruit from NaCl solution gave a faint positive reaction for peroxidase and a moderately pronounced test for organic peroxide. Negative tests for organic peroxide were obtained with fruit from the HCl and  $Na_2SO_3$  solutions.

Sliced fruit was immersed in the solutions for five minutes and fifteen minutes, respectively, and then exposed to the air for forty-two

hours.  $\text{HCl}$  and  $\text{Na}_2\text{SO}_3$  prevented browning;  $\text{NaCl}$ , cane sugar, and  $\text{NaNO}_3$  reduced, but did not prevent browning. Fruit treated with  $\text{HCl}$  and  $\text{Na}_2\text{SO}_3$  gave negative reactions for organic peroxide. When treated with  $\text{NaCl}$  it gave weaker positive reactions for both peroxidase and organic peroxide than did the untreated check. It, therefore, appears that  $\text{NaCl}$  may reduce the activity of both the peroxidase and organic peroxide.

Sodium chlorid was added to samples of fresh apple juice to give concentrations varying from 0.35% to 15%  $\text{NaCl}$ . The juice darkened within twelve hours in samples containing less than 2.5%  $\text{NaCl}$ , but did not darken with concentrations above 3%  $\text{NaCl}$ . The intensity of darkening decreased with increase in  $\text{NaCl}$  concentration. Darkening was checked temporarily with the lowest concentration of  $\text{NaCl}$  employed.

The tests indicated that the darkening of apples during drying might be prevented by dipping in a 3% or more  $\text{NaCl}$  solution. Hence, apples were peeled, sliced, and immersed for one-half to five minutes in 5%  $\text{NaCl}$  solution and dried at  $135^\circ \text{F}$ . The dried product was much lighter in color than the untreated fruit dried at the same temperature—as light as most commercial dried apples—but was not so light as fruit from the same lots sulfured for thirty minutes before drying. Fruit dipped in dilute brine (3 to 5%) required a shorter exposure to fumes of burning sulfur than fruit not previously treated with  $\text{NaCl}$  solution.

Tests were made to determine whether the inhibiting action of  $\text{NaCl}$  was due to its action on the coloring matter in the apple. Several 10 c.c. portions of juice were treated, as shown in table 10. Lot No. 1 was freshly pressed untreated juice; No. 2, juice pressed from boiled sliced apples; No. 3, juice from sliced apples sulfured thirty minutes and boiled in water, and No. 4, sulfured apples.

After an excess of  $\text{H}_2\text{O}_2$  had been added to juice lacking a complete oxidase system,  $\text{NaCl}$  did not prevent darkening. This might indicate that  $\text{NaCl}$  prevents or reduces darkening by its action on the peroxidase or organic peroxide rather than upon the coloring matter.

Rose, Kraybill and Rose's<sup>25</sup> experiments to determine the effect of various salts upon the oxidase of apple bark were repeated with the juice of Yellow Newtown apples. To minimize oxidation changes



during the interval between the extraction of the juice and the addition of the salts, the fruit was crushed and pressed in a cold-storage room at 0° C. Measured quantities of N/1 solutions of the salts were added to test tubes, so that, after bringing the total volumes to 10 c.c. by the addition of fresh juice, concentrations of

TABLE 10  
EFFECT OF NaCl ON COLORING MATTER OF APPLE JUICE

Test No.	Juice	Treatment	Color after 24 hours
1	Untreated	No treatment	Dark brown
2	Boiled juice	No treatment	Light color
3	Boiled sulfured	No treatment	Light color
4	Sulfured	No treatment	Light color
5	Untreated	One c.c. H <sub>2</sub> O <sub>2</sub> solution per 10 c.c.	Dark brown but lighter than No. 1
6	Boiled	One c.c. H <sub>2</sub> O <sub>2</sub> solution per 10 c.c.	Faint darkening of color
7	Boiled sulfured	One c.c. H <sub>2</sub> O <sub>2</sub> solution per 10 c.c.	Faint darkening of color
8	Sulfured	One c.c. H <sub>2</sub> O <sub>2</sub> solution per 10 c.c.	Slight darkening of color Darker than 6 or 7. Lighter than No. 5
9	Untreated	NaCl 10% after juice had browned in color	No increase in darkening
10	Boiled	NaCl 10%	No change
11	Boiled sulfured	NaCl 10%	No change
12	Sulfured	NaCl 1%	No change
13	Untreated	NaCl 10%, plus 1% H <sub>2</sub> O <sub>2</sub> after solution had browned	Color same as No. 5
14	Boiled	NaCl 10%, plus 1% H <sub>2</sub> O <sub>2</sub> solution	Color same as No. 6
15	Boiled sulfured	NaCl 10%, plus 1% H <sub>2</sub> O <sub>2</sub> solution	Color same as No. 7
16	Sulfured	NaCl 10%, plus 1% H <sub>2</sub> O <sub>2</sub> solution	Color same as No. 8

N/10 and N/100 were obtained for each of the salts. The samples were removed to room temperature (16° to 20° C.), and the rapidity and intensity of browning compared with that occurring in untreated samples.

The hydrogen-ion concentration of each sample was determined by means of a hydrogen electrode apparatus, to determine whether correlation existed between the inhibition of browning by certain salts and

their effect upon the hydrogen-ion concentration of the juice. The determinations were made at 20° C., using a N/1 KCl-calomel electrode with a tube filled with saturated KCl in agar jelly to connect the solution to be treated and the calomel electrode. Hydrogen from a cylinder of the gas was used to saturate the hydrogen electrode. A voltmeter and a delicate galvanometer were used to make determinations of  $E_{\frac{n}{1}}$ , i.e., the voltage given by the solution tested. From the tables of Schmidt and Hoagland<sup>26</sup> the corresponding pH values were found. With pure water the reading is as follows:  $E_{\frac{n}{1}}$ —0.697 and pH —6.999. Values of pH less than 6.999 indicate an "acid" condition, and values greater than 6.999 an "alkaline" condition of the solution.

Tables 11 to 14, inclusive, summarize the data on the effect of various neutral salts in preventing darkening and in changing the pH value of the juice.

Tenth normal solutions of the chlorids almost prevented browning. Hundredth normal solutions slightly retarded, but did not prevent it. Chlorids slightly increased the hydrogen-ion concentration of the juice, but not sufficiently to explain entirely their inhibitory action.

Sulfates were apparently without effect, with the exception of potassium aluminum sulfate, which, in N/10 concentration, however, appreciably retarded the darkening process, and also increased the hydrogen-ion concentration of the juice.

Nitrates noticeably increased the rate of browning, probably because of their oxidizing character.

Acetates and tartrates did not affect the rate of browning of apple juice, but the oxalate exerted a powerful inhibitory action in N/10 and N/100 concentrations. As this salt reduced the hydrogen-ion concentration (a pH value of 4.243 in N/10 solution compared to 3.347 for untreated juice), its inhibitory action cannot be explained as due to an increase of the hydrogen-ion concentration. Reactions for organic peroxide were negative in juice containing ammonium oxalate. The test for peroxidase was positive. It appeared, therefore, that the oxalate prevented darkening by its action upon the organic peroxide. In this respect it resembled sulfurous acid. Oxalic acid behaved similarly to ammonium oxalate in its action on the organic peroxide.



TABLE 11  
EFFECT OF N/10 AND N/100 SOLUTIONS OF CHLORIDS UPON THE DARKENING AND pH VALUE OF APPLE JUICE

Solution	Color after 10 minutes		Color after 5 hours		pH Value	
	N/10 Solution	N/100 Solution	N/10 Solution	N/100 Solution	N/10 Solution	N/100 Solution
NH <sub>4</sub> Cl	No change	Very faint brown	Very faint brown	Marked brown; less than check	3.111	3.178
BaCl <sub>2</sub>	No change	Very faint brown	Very faint brown	Marked brown; less than check	3.009	3.178
CaCl <sub>2</sub>	No change	Very faint brown	Very faint brown	Marked brown; less than check	3.18	3.32
LiCl	No change	Very faint brown	Very faint brown	Marked brown; less than check	3.32	3.43
MgCl <sub>2</sub>	No change	Very faint brown	Very faint brown	Marked brown; less than check	3.31	3.45
KCl	No change	Very faint brown	Very faint brown	Marked brown; less than check	3.31	3.45
NaCl	No change	Very faint brown	Very faint brown	Marked brown; less than check	3.31	3.46
NaCl					$\frac{N}{1} 2.98$	
NaCl Check	Marked brown	Marked brown	Very marked brown	Very marked brown	2N 2.89 3.50	

TABLE 12  
EFFECT OF N/10 AND N/100 SOLUTIONS OF SULFATES UPON THE DARKENING AND pH VALUE OF APPLE JUICE

Solution	Color after 10 minutes		Color after 5 hours		pH Value	
	N/10 Solution	N/100 Solution	N/10 Solution	N/100 Solution	N/10 Solution	N/100 Solution
*K <sub>2</sub> SO <sub>4</sub>	Faint brown	Faint brown	Appreciable brown; not so brown as check	About same as check	3.246	3.415
Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	Faint brown	Faint brown	Marked brown	About same as check	3.550	3.50
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Faint brown	Faint brown	Marked brown	About same as check	3.415	3.483
MgSO <sub>4</sub>	Faint brown	Faint brown	Marked brown	About same as check	3.590	3.516
K <sub>2</sub> SO <sub>4</sub>	Faint brown	Faint brown	Marked brown	About same as check	3.50	3.50
NaSO <sub>4</sub>	Marked brown	Marked brown	Marked brown	Marked brown	3.50	3.50
Check						

\* After 48 hours N/10 alum [K<sub>2</sub>SO<sub>4</sub>, Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>] solution was very much lighter than check.

TABLE 13  
EFFECT OF N/10 AND N/100 SOLUTIONS OF NITRATES UPON THE DARKENING AND pH VALUE OF APPLE JUICE

Solution	Color after 10 minutes		Color after 5 hours		pH Value
	N/10 Solution	N/100 Solution	N/10 Solution	N/100 Solution	
(NH <sub>4</sub> )NO <sub>3</sub>	Faint brown	Faint brown	Marked brown, darker than check	Same as check	3.32
Ca (NO <sub>3</sub> ) <sub>2</sub>	Faint brown	Faint brown	Marked brown, darker than check	Marked brown	3.32
KNO <sub>3</sub>	Faint brown	Faint brown	Marked brown, darker than check	Same as check	3.32
Mg(NO <sub>3</sub> ) <sub>2</sub>	Faint brown	Faint brown	Marked brown, darker than check	Marked brown	3.32
NaNO <sub>3</sub>	Faint brown	Faint brown	Marked brown, darker than check	Same as check	3.32
Ba(NO <sub>3</sub> ) <sub>2</sub>	Faint brown	Faint brown	Marked brown, darker than check	Marked brown	3.32
Check	Faint brown	Faint brown	Marked brown	Same as check	3.50

TABLE 14  
EFFECT OF N/10 AND N/100 SOLUTIONS OF VARIOUS ORGANIC SALTS UPON THE DARKENING AND pH VALUE OF APPLE JUICE

Solution	Color after 10 minutes		Color after 5 hours		pH Value
	N/10	N/100	N/10	N/100	
Na (H <sub>3</sub> C <sub>2</sub> O <sub>2</sub> ) acetate	Appreciably brown	Appreciably brown	Very marked brown	Very marked brown	3.88
NaK (C <sub>4</sub> H <sub>4</sub> O <sub>6</sub> ) tartrate	Appreciably brown	Appreciably brown	Very marked brown	Very marked brown	3.52
(NH <sub>4</sub> ) <sub>2</sub> C <sub>2</sub> O <sub>4</sub> oxalate	No change	No change	No change	No change	4.429
NH <sub>4</sub> (H <sub>3</sub> C <sub>2</sub> O <sub>2</sub> ) acetate	Appreciably brown	Appreciably brown	Very marked brown	Very marked brown	3.922-Check 3.50
Check	Marked brown	Marked brown	Very marked brown	Very marked brown	4.243
					3.347

*Electrolysis.*—In one experiment, 500 c.c. of boiled non-darkened apple juice was placed in a beaker. A large alundum Soxhlet extraction thimble containing juice and a small platinum dish attached to the positive pole of an Edison storage battery were immersed in the beaker. A platinum-wire spiral connected to the negative pole of the battery was inserted in the thimble. The dish served as anode and the wire as cathode. The thimble, because of its porous nature, permitted migration of ions but prevented rapid mixing of the solutions around the two electrodes.

After twenty hours' electrolysis, the juice at the anode had darkened noticeably in comparison with untreated boiled juice. The juice at the negative pole was distinctly alkaline in reaction and dark brown, but the color disappeared upon acidification, indicating that the browning at this pole was caused by the alkaline reaction of the juice. The juice at the anode was distinctly acid in reaction. Therefore, darkening at this pole was not due to alkalinity, but to oxidation by nascent oxygen liberated by electrolysis.

After forty-eight hours the juice at the anode had darkened more than at twenty hours. The cathode juice was strongly alkaline; 10 c.c. required 16 c.c. of N/10 HCl for neutralization; the specific gravity was 1.026. For neutralization of 10 c.c. of the anode juice of 1.02 specific gravity 3.2 c.c. N/10 NaOH were required. The cathode juice became alkaline, because of the migration of metallic ions to this electrode. The cathode vessel (alundum thimble) contained only one-sixth the volume of juice contained in the beaker in which the anode was inserted. The test was repeated with similar results. Electrolysis of sulfured juice gave an alkaline and dark brown liquid at the cathode. The brown color disappeared upon acidification. The sulfured juice did not darken at the anode, probably because of the presence of  $\text{H}_2\text{SO}_3$  or  $\text{H}_2\text{SO}_4$ .

In another experiment a dilute solution of tannic acid (less than 1%) containing 0.12% citric acid was electrolyzed as described for apple juice. The liquid in the cathode vessel became alkaline in reaction and somewhat turbid, but remained light in color; 10 c.c. of this liquid neutralized 1.2 c.c. N/10 HCl. The liquid at the anode after seventy-two hours became dark brown but showed no turbidity; 10 c.c. neutralized 1 c.c. N/10 NaOH.

From these results the following conclusions were drawn:

1. Boiled apple juice contains a compound which darkens in consequence of oxidation by electrolysis.

2. It contains a compound which becomes brown in color when the juice at the cathode is made alkaline by electrolysis, but which is not identical with ordinary tannic acid. It is possible that the brown color may be the effect of alkali upon organic matter other than tannic acid.

3. The compound in apple juice which darkens at the anode resembles tannic acid in this respect.

4. The compound in apple juice which becomes brown in alkaline solution and colorless in acid solution may differ from the one that darkens in consequence of oxidation.

*Function of Sulfurous Acid in Preventing Darkening of Apples.*—In the presence of water, sulfur dioxide bleaches many organic coloring matters. This action may in some cases depend upon the union of the color with the sulfur dioxide, because the color returns if the juice is warmed and the gas expelled. In other cases, the bleaching action apparently depends upon the abstraction of oxygen from the coloring matter. We suggest that sulfurous acid acts as a reducing agent in preventing the browning of fruits; also that the formation of sulfuric acid by oxidation of sulfurous acid might affect the color production because of an increase in hydrogen-ion concentration.

Some workers consider that the sulfur dioxide used in drying fruit combines with the tannin or coloring matter and thus prevents darkening by oxidation. Brown<sup>6</sup> apparently accepts this hypothesis, for he refers to the sulfuring process as "bleaching or color setting." Sulfurous acid in preventing darkening may affect: (a) chromogen, or coloring matter; (b) enzymes or the peroxidase and organic peroxide.

(a) It has been assumed by some investigators<sup>2</sup> that darkening is prevented by the action of the sulfurous acid on the coloring matter. Subjecting the tissue to  $\text{SO}_2$  for prolonged periods of time, however, does not modify the result of adding to the juice those reagents that give characteristic reactions for the tannins.

(b) Efforts were made to determine whether  $\text{SO}_2$  destroyed the peroxidase or the organic peroxide, in preventing darkening of apple tissue. Fresh Newtown apples were sliced and left for forty-eight hours in sealed jars from which the air had been displaced by  $\text{SO}_2$ .



The slices were then scarified and tested for peroxidase and organic peroxide, with the following results: (1) Tests for oxidase with benzidine and guaiac without  $\text{H}_2\text{O}_2$  were negative. (2) Test with benzidine and guaiac plus  $\text{H}_2\text{O}_2$  were positive. (3) Tests for organic peroxide with a solution of potassium iodide, starch and acetic acid were negative. The results indicated that the  $\text{SO}_2$  did not destroy the oxidase, but that it affected the organic peroxide.

TABLE 15

RESPONSE OF SLICED SULFURED NEWTOWN APPLES TO VARIOUS REAGENTS

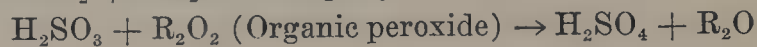
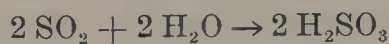
Treatment	Results
Aqueous solution of benzidine 1%	Negative, no blue oxidation color
Tincture of guaiac	Negative, no blue oxidation color
Benzidine (1%) plus $\text{H}_2\text{O}_2$	Positive test for peroxidase; marked blue oxidation color
Tincture of guaiac plus $\text{H}_2\text{O}_2$	Positive test for peroxidase; characteristic oxidation blue color
Check. No treatment except tissue scarified	Negative. No color development
Dilute KI plus dilute acetic plus 1% starch sol. added without $\text{H}_2\text{O}_2$	Negative. No test for peroxide
Sulfured scarified tissue to which $\text{H}_2\text{O}_2$ was added	Positive; a marked browning of tissue similar to that from normal oxidation process
Sulfured tissue washed in running $\text{H}_2\text{O}$ 15 hours	Positive test for peroxidase with tincture of guaiac, and strong positive test for peroxide with KI starch-acetic acid solution
Tincture guaiac also KI solution starch-acetic acid	

Slices of sulfured apples placed in 3%  $\text{H}_2\text{O}_2$  darkened more rapidly than did freshly cut untreated tissue. The sulfured tissue when placed in water, or when boiled and placed in hydrogen peroxide, did not darken. Boiling destroyed the enzyme (peroxidase).

Tests were repeated on apple tissue from whole specimens that had been kept in  $\text{SO}_2$  for three months. This tissue gave positive reactions for peroxidase but negative tests for organic peroxide.

Further tests were conducted to substantiate the theory that  $\text{SO}_2$  prevents the darkening of Newtown apples by reacting with the organic peroxide rather than with the enzyme. The apples were sulfured by the usual commercial method—slices about  $\frac{3}{8}$  inch thick were exposed to the fumes of burning sulfur for 25 minutes. Tests were then conducted, as summarized in table 15.

Sulfured tissue did not give a positive test for the oxidizing enzyme without the addition of  $\text{H}_2\text{O}_2$ . This would indicate that the  $\text{SO}_2$  removed the organic peroxide; or, more probably, that sulfurous acid removed the extra oxygen atom from the organic peroxide. The following equations can be employed to express the latter viewpoint:



According to the definition of terms previously made, we consider that a negative test without  $\text{H}_2\text{O}_2$  and a positive test with  $\text{H}_2\text{O}_2$  indicates the absence of an organic peroxide and the presence of a peroxidase. This view is substantiated by the fact that when a positive reaction for peroxidase can be obtained without the addition of  $\text{H}_2\text{O}_2$ , a positive reaction for an organic peroxide is also obtained, and that no positive test for the organic peroxide can be obtained when the addition of  $\text{H}_2\text{O}_2$  is necessary.

The result of one experiment indicated that it was possible by prolonged washing with running water to remove the sulfurous acid and subsequently to demonstrate the presence of the peroxidase and the organic peroxide. This fact may mean (a) that the  $\text{SO}_2$  and the peroxide were in a loose combination which was broken up by the washing and dialysis; or (b) that the water merely removed the  $\text{SO}_2$ , and that the organic peroxide was again formed by the tissue freed of  $\text{SO}_2$ .

A positive reaction for peroxidase can be obtained in sulfured tissue; no organic peroxides are found, and sulfured tissue plus  $\text{H}_2\text{O}_2$  reacts positively by an apparently normal browning. Therefore, it would seem that  $\text{SO}_2$  prevents darkening by combining with the organic peroxide rather than by destroying or combining with the enzymes or the chromogens of the fruit.

*Permanency of Effect of Sulfurous Acid.*—Apples sulfured and dried in the usual commercial way in the fall of 1917 were tested for enzymes and organic peroxides in March, 1919. Slices were soaked in water for fifteen minutes and tested for oxidase with benzidine and with guaiac, without addition of  $\text{H}_2\text{O}_2$ . A slight positive reaction for the enzyme was obtained in each instance. Another test, with the addition of  $\text{H}_2\text{O}_2$ , was made. A slight positive reaction for peroxidase and a strong positive reaction for peroxide were obtained.

The apples at the top of the box had browned appreciably, whereas those at the bottom were of a desirable light color. This indicated that the exposure to the air of the apples in the upper layer of the box permitted oxidation.

This experiment substantiates the theory that in the process of drying the peroxidase is not destroyed but may remain potentially active for a considerable time. It also indicates that the organic peroxide may be re-formed. This may account for the darkening of dried apples that are exposed to the air for long periods.

TABLE 16

EFFECT OF SULFURIC ACID UPON OXIDIZING ENZYME AND ORGANIC PEROXIDE OF YELLOW NEWTOWN APPLES

Reagents used	Tests for oxidase by bluing of 2% benzidine, after 1 hour immersion	Test for oxidase by bluing of 2% benzidine, after 48 hours immersion	Test for peroxide with KI-starch-acetic acid solution, after 48 hours immersion
5% H <sub>2</sub> SO <sub>4</sub>	Negative	Negative with 2% benzidine alone or plus H <sub>2</sub> O <sub>2</sub>	Positive
H <sub>2</sub> O	Positive	Positive	Positive
Boiled tissue	Negative	Negative	Negative
Untreated tissue	Positive	Positive	Positive

*Acids other than Sulfurous.*—Experiments were conducted to determine the effects of acids other than sulfurous upon the browning of apple tissue and juice. When a noticeable effect was obtained, attempts were made to determine whether it was due to the influence of the acid on the peroxidase, on the peroxide, or on both.

Slices of freshly cut Newtown apples were dropped in separate portions of the acid solutions and left for varying periods. Slices were placed in tap water as checks. Observations were made on the amount and rate of darkening. The slices were also tested for peroxidase and organic peroxide. Some of the data obtained are summarized in table 16.

Slices of apples immersed for one hour in 5% H<sub>2</sub>SO<sub>4</sub> failed to cause the bluing of benzidine alone or benzidine and H<sub>2</sub>O<sub>2</sub>. A positive reaction for peroxide, however, was obtained when the tissue was immersed for forty-eight hours. The 5% H<sub>2</sub>SO<sub>4</sub> destroyed the peroxidase, but not the peroxide, thus indicating that H<sub>2</sub>SO<sub>4</sub> resulting from the oxidation of SO<sub>2</sub> in sulfured fruit may affect the peroxidase.

TABLE 17  
EFFECT OF HYDROGEN-ION CONCENTRATION AS INFLUENCED BY DIFFERENT ACIDS UPON THE BROWNING OF NEWTOWN APPLE JUICE

Observation on browning at room temperature at following intervals																	Hrs.	Total N/5 HCl present
pH	MINUTES																	
	3	12	18	24	30	36	42	50	60	66	72	82	120	145	165	48		
3.516	v.f.	app.	mark	mark	mark	mark	mark	mark	mark	mark	mark	mark	mark	mark	mark	mark	none	
3.398	none	v.f.	app.	mark	mark	mark	mark	mark	mark	mark	mark	mark	mark	mark	mark	mark	1 c.c.	
3.313	none	none	v.f.	v.f.	app.	n.m.	mark	mark	mark	mark	mark	mark	mark	mark	mark	mark	2 c.c.	
2.246	none	none	none	none	v.f.	app.	n.m.	app.	mark	mark	mark	mark	mark	mark	mark	mark	3 c.c.	
2.982	none	none	none	none	none	v.f.	v.f.	f.	n.m.	n.m.	mark	mark	mark	mark	mark	mark	4 c.c.	
2.840	none	none	none	none	none	none	none	none	app.	app.	mark	mark	mark	mark	mark	mark	5 c.c.	
2.755	none	none	none	none	none	none	none	none	v.f.	v.f.	n.m.	mark	mark	mark	mark	mark	6 c.c.	
2.671	none	none	none	none	none	none	none	none	none	none	app.	mark	mark	mark	mark	mark	7 c.c.	
2.586	none	none	none	none	none	none	none	none	none	none	f.	mark	mark	mark	mark	mark	8 c.c.	
2.519	none	none	none	none	none	none	none	none	none	none	v.f.	app.	n.m.	mark	mark	mark	9 c.c.	
2.282	none	none	none	none	none	none	none	none	none	none	none	none	app.	n.m.	mark	mark	10 c.c.	
2.215	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	1 c.c. N/1	
	3	6	12	18	24	30	36	40	46								4 c.c. N/1	
																	Total	
																	H <sub>2</sub> SO <sub>4</sub> present	
3.618	v.f.	f.	app.	n.m.	mark	mark	mark	mark	mark								Check	
3.127	none	v.f.	f.	app.	n.m.	n.m.	mark	mark	mark	mark							0.1 c.c. N/1	
2.519	none	none	v.f.	f.	app.	v.f.	v.f.	v.f.	v.f.	v.f.							0.3 c.c. N/1	
1.961	none	none	none	none	none	none	none	none	none	none							0.5 c.c. N/1	
1.657	none	none	none	none	none	none	none	none	none	none							1.0 c.c. N/1	
1.369	none	none	none	none	none	none	none	none	none	none							2.0 c.c. N/1	
	3	6	9	12	15	18	21	24	27	30							Total Citric	
																	Acid present	
3.665	v.f.	f.	app.	n.m.	mark	mark	mark	mark	mark	mark							Check	
3.381	none	v.f.	f.	app.	n.m.	mark	mark	mark	mark	mark	mark						0.1 c.c. N/1	
3.330	none	v.f.	v.f.	n.ap.	app.	n.m.	mark	mark	mark	mark	mark						0.3 c.c. N/1	
3.161	none	none	v.f.	f.	n.ap.	app.	n.m.	mark	mark	mark	mark						0.5 c.c. N/1	
2.958	none	none	none	v.f.	f.	n.ap.	app.	n.m.	mark	mark	mark						1.0 c.c. N/1	
2.654	none	none	none	none	v.f.	f.	n.ap.	app.	n.m.	mark	mark						2.0 c.c. N/1	
	3	6	9	12	15	18	21	24	27	30							Total Acetic	
																	Acid present	
3.665	v.f.	f.	app.	n.m.	mark	mark	mark	mark	mark	mark							Check	
3.516	none	v.f.	f.	app.	n.m.	mark	mark	mark	mark	mark	mark						0.1 c.c. N/1	
3.483	none	v.f.	f.	n.ap.	app.	n.m.	mark	mark	mark	mark	mark						0.3 c.c. N/1	
3.381	none	none	v.f.	f.	n.ap.	app.	n.m.	mark	mark	mark	mark						0.5 c.c. N/1	
3.296	none	none	none	v.f.	f.	n.ap.	app.	n.m.	mark	mark	mark						1.0 c.c. N/1	
3.144	none	none	none	none	v.f.	f.	n.ap.	app.	n.m.	mark	mark						2.0 c.c. N/1	

Abbreviations: v.f.—very faintly; f.—faintly; n.ap.—nearly appreciably; app.—appreciably; n.m.—nearly marked; mark—markedly browned.



Fresh tissue in 5%  $\text{H}_2\text{SO}_4$  remained whitish in color and did not darken by oxidation. Boiled tissue untreated by the solutions behaved similarly. The tissue, immersed in water or left untreated, browned.

*Hydrogen-ion Concentration.*—Falk and co-workers<sup>10</sup> found that the hydrogen-ion concentration of the medium has a marked effect on the activity of peroxidase. They state that the optimum hydrogen-ion concentration is  $10^{\frac{-6}{n}}$  to  $10^{\frac{-10}{n}}$  and that peroxidase is inactivated at a concentration of  $10^{\frac{-2}{n}}$  to  $10^{\frac{-4}{n}}$ .

We conducted experiments in which the reaction of apple juice was influenced by the addition of various acids and alkalies. The effect of these reagents upon the color changes of the juice and upon the peroxidase and peroxide were noted, and determinations of the hydrogen-ion concentration were made. The tests were undertaken primarily to determine if there is any relation between the inhibiting effect of sulfurous acid upon the darkening of apple tissue and its increase of hydrogen-ion concentrations.

In order to obtain a sample in which browning had not occurred during crushing and pressing, the juice was extracted in a cold-storage room at  $0^\circ \text{C}$ . It was found by chilling all the equipment and material to  $0^\circ \text{C}$ ., by having in the test tubes the required amounts of acids, and by quickly adding the expressed juice, that the entire series could be prepared without appreciable darkening.

The strength of the  $\text{HCl}$  was varied by adding 1 c.c. of  $\text{HCl}$  of known normality and 9 c.c. of distilled water to the first tube, and in the second, 2 c.c. of the  $\text{HCl}$  solution and 8 c.c. of water. The last tube in the series contained 10 c.c. of the  $\text{HCl}$  solution only. Ten cubic centimeters of apple juice were added to each tube, so that the juice in all was of the same dilution. The samples were then removed from cold storage and observations taken to determine the rate of darkening. The data are summarized in table 17.

#### SUMMARY AND CONCLUSIONS

1. The oxidizing system concerned in the browning of apple tissue has been considered as consisting of a peroxidase and an organic peroxide. The peroxidase transfers to the compound to be oxidized the oxygen derived from the organic peroxide occurring in the fruit,

or that derived from added  $\text{H}_2\text{O}_2$ , and behaves as an activator or accelerator. The organic peroxide is considered to resemble hydrogen peroxide in its behavior; in the presence of the peroxidase it liberates oxygen in the active state.

2. Benzidine (1% aqueous solution) is an excellent indicator for peroxidase in apple tissue or apple juice when used with  $\text{H}_2\text{O}_2$  and is preferable to tincture of guaiac, to dilute solutions of guaiacol, alpha-naphthol, phenol, and several other reagents. With the apple, benzidine alone is also satisfactory.

3. A dilute solution of potassium iodide, starch, and acetic acid proved satisfactory in determining qualitatively organic peroxide in apple tissue or juice. The ferrous sulfate solution usually employed in this test was unsatisfactory, because of the color reaction of iron salts with apple tannin.

4. The organic peroxide and peroxidase were separated from the tannin of apple juice by precipitation of the enzymes with 95% alcohol. Browning of the tannin solution freed from alcohol did not occur until a solution of the enzyme precipitate containing the peroxidase and organic peroxide was added. These results indicate that apple tannin and organic peroxide are separate entities.

5. Boiled apple juice did not darken on addition of various oxidizing reagents, but did so when the acidity was reduced to near the neutral point and  $\text{MnO}_2$ , or platinizing solution, and  $\text{H}_2\text{O}_2$  were added.

6. Qualitative tests made to determine the character of apple tannin indicated that it belongs to the catechol group.

7. Treatment of whole apples with nitrous ether gave uniform browning throughout the tissue, thus indicating the even distribution of the tannin.

8. Heating apple tissue and apple juice to temperatures ranging from  $56^\circ\text{C}$ . to  $100^\circ\text{C}$ . demonstrated that the organic peroxide is much more susceptible to heat than is the peroxidase. The former was inactivated at from  $73.5^\circ$  to  $78^\circ\text{C}$ ., and the latter between  $90^\circ$  and  $100^\circ\text{C}$ . Re-formation of the peroxide did not occur in the heated tissue or juice within forty-eight hours.

9. Apples stored in nitrogen for one month remained unaltered in appearance; they also remained normal in appearance after removal from the gas and gave positive reactions for peroxidase and organic peroxide. Apples after being stored in  $\text{CO}_2$ ,  $\text{H}_2$  or  $\text{O}_2$  browned



rapidly, and their tissues gave negative reactions for organic peroxide. It appeared that such gases increase the permeability of the cells, permitting a mixing of their contents with the enzymes, with resultant darkening of color and utilization of the oxygen of the organic peroxide. It is possible that nitrogen may be used for the storage of fruit on a commercial scale.

10. Vapors of carbon bisulfid, formaldehyde, carbon tetrachlorid, benzine, ether, gasoline, and ethyl alcohol were used for storage of whole apples, with results similar to those indicated as having been obtained with  $\text{CO}_2$ ,  $\text{O}_2$ , and  $\text{H}_2$ .

11. Immersion in 5% solutions of  $\text{NaCl}$ ,  $\text{HCl}$ ,  $\text{Na}_2\text{SO}_3$ , or cane sugar for three days prevented darkening of slices of Yellow Newtown apples, even after removal from these solutions. When slices of Yellow Newtown apples were immersed in tap water, and in 5% solutions of  $\text{NaNO}_2$ ,  $\text{NaNO}_3$ , or  $\text{NaCO}_3$ , darkening occurred.

12. Solutions containing 5%  $\text{HCl}$  and 5%  $\text{Na}_2\text{SO}_3$  prevented subsequent browning of the tissue when slices were immersed for five minutes;  $\text{NaCl}$ , cane sugar, or  $\text{NaNO}_3$  reduced but did not prevent subsequent browning. The  $\text{HCl}$  and  $\text{Na}_2\text{SO}_3$  appeared to prevent the browning of sliced apple tissue by destroying the organic peroxide. The action was comparable in this respect to that of  $\text{SO}_2$ , and in all probability the effect of the  $\text{Na}_2\text{SO}_3$  was due to  $\text{H}_2\text{SO}_3$  formed in the apple tissue.

13. A solution of  $\text{NaCl}$  apparently lessened or checked browning by inhibiting activity of the peroxidase and the peroxide, but did not destroy either.

14. Slices of apple tissue in  $\text{NaNO}_2$  and  $\text{Na}_2\text{CO}_3$  became alkaline in reaction. This condition apparently favored rapid darkening by oxidation and by effect on the chromogen, which is brown when alkaline and colorless when acid.

15. Laboratory tests indicated that 3% to 5%  $\text{NaCl}$  solutions gave a satisfactory dried apple product, although the color was not so light as was obtained with sulfured lots. However, fruit dipped in 3% to 5% brine required much less exposure to sulfur fumes than the non-dipped fruit.

16. Browning of freshly extracted apple juice was almost prevented by N/10 solutions of the following:  $\text{NH}_4\text{Cl}$ ,  $\text{BaCl}_2$ ,  $\text{CaCl}_2$ ,  $\text{LiCl}$ ,  $\text{MgCl}_2$ ,  $\text{KCl}$ , and  $\text{NaCl}$ ; N/100 solutions slightly retarded but did not

prevent browning of the juice. All of the chlorids slightly increased the hydrogen-ion concentration when added to the juice, but it seems unlikely that their inhibitory action upon the browning can be explained on this basis.

17. With the exception of potassium aluminum sulfate, neither N/100 nor N/10 solutions of sulfates had any effect in checking browning of freshly extracted apple juice. The alum salt appreciably retarded the darkening process, and slightly increased the hydrogen-ion concentration of the juice.

18. Nitrates of N/10 and N/100 concentrations increased the rate of browning. This might be explained on the basis of the oxidizing character of the nitrates. Acetates and tartrates of similar concentrations had no appreciable effect on the rate of browning. The oxalate, both in N/10 and N/100 concentrations, exerted a marked inhibitory action upon browning of fresh apple juice.

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JULY, 1923

TECHNICAL PAPER No. B

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EFFECT OF SALTS ON THE INTAKE OF  
INORGANIC ELEMENTS AND ON THE  
BUFFER SYSTEM OF THE PLANT

BY

D. R. HOAGLAND AND J. C. MARTIN

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EFFECT OF SALTS ON THE INTAKE OF  
INORGANIC ELEMENTS AND ON THE  
BUFFER SYSTEM OF THE PLANT

BY

D. R. HOAGLAND AND J. C. MARTIN

(Contribution from the Division of Plant Nutrition, University of California)

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INTRODUCTION

In arid regions various types of injury to plants have been associated with the presence of alkali\* salts in the soil. While many observations have been made in the field for the purpose of determining the tolerance of different plants to alkali conditions, comparatively few controlled experiments have been carried out with intent to study the effect of an alkali soil or solution on the chemical system of the plant. Information of this character, however, is essential for an understanding of the relation of the plant to the medium in which it grows. Only by means of intensive studies made on the plant itself will it be possible to reach definite conclusions concerning the nature of the injury produced by different salts or to explain the varying responses of different plants growing in the same soil or solution. A systematic investigation of these questions is being attempted in this laboratory and certain data bearing on one phase of the work are reported in the present article.

Previous studies of the absorption of inorganic elements by barley plants suggested that it would be of considerable interest to ascertain how sodium salts influence the absorption of important ions from

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\* The term 'alkali' is used in its general and customary sense, but it is well to note that the expression is technically inaccurate. As our knowledge advances, it will be desirable to substitute other more exact expressions, such as salinity, alkalinity, and appropriate sub-classifications.

culture\* solutions. Both sand and solution cultures were employed in the course of the investigations. In one group of experiments, the absorption studies were conducted for very short periods and the solutions were analyzed. In another group the plants were grown for longer periods in the solutions under examination and were themselves analyzed. In one instance, the plants were grown to complete maturity in sand cultures.

Data on the intake of ions by the plant are often of value to the investigator interested in soil and plant relations, but it is evident that certain limitations may have to be placed on the interpretation of such data. For example, Osterhout<sup>1</sup> and Brooks<sup>2</sup> have pointed out that the rate of absorption of an ion is not necessarily a measure of cell permeability. Various reactions occurring in the cell, on surfaces, or in intercellular spaces may cause the ion to be removed from the influence of diffusion equilibria, with the result that considerable absorption may take place even when the cell possesses a low degree of permeability. It may be noted that a somewhat different conception of permeability has been presented by Stiles and Jorgensen.<sup>3</sup> In the present investigation no attempt is made to interpret the results in terms of cell permeability.

#### EFFECT OF SALTS ON ABSORPTION OF IONS

The technique of the first experiments described consisted in growing large numbers of barley plants during a period of two to three weeks in a complete culture solution† and then dividing the cultures into uniform sets. Each set consisted of either 49 or 98 plants, duplicate sets being used in a number of experiments. After rinsing the roots with distilled water, the plants were transferred to the various solutions that were to be examined. The culture vessels were tumblers of about 110 c.c. capacity, each provided with a cork stopper bored with seven holes. At the conclusion of the experiment the solutions were removed from the plants, made up to volume, and analyzed. Water was added as required during the period of the

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\* The expressions 'culture solutions' and 'nutrient solutions' are used interchangeably in this paper, according to custom, but the latter term cannot be considered to be strictly accurate.

† Similar to that used in previous nutrition studies. Composition given in table 1.

TABLE 1.—EFFECT OF NaCl AND Na<sub>2</sub>SO<sub>4</sub> ON THE ABSORPTION OF NUTRIENTS IN SOLUTION CULTURE

Description of Solution	Salt added		K R.V.	Ca R.V.	Mg R.V.	NO <sub>3</sub> R.V.	(H <sub>2</sub> ) PO <sub>4</sub> R.V.	SO <sub>4</sub> R.V.	H <sub>2</sub> O Absorbed c.c.
	R. V.	P. P. M. Na							
<i>Experiment A—3-Day Period</i> (October 29—November 1)									
Composition of nutrient solution.....			4.35	8.55	4.10	11.2	1.16	4.12	
Absorption from nutrient solution.....			3.43	1.15	.33	7.5	.29	.33	900
* Absorption from nutrient solution plus NaCl.....	34.8	800	2.12	.90	.25	7.7	.28	.44	800
* Absorption from nutrient solution plus Na <sub>2</sub> SO <sub>4</sub> .....	34.8	800	2.23	.85	.14	8.7	.27		900
<i>Experiment B—3-Day Period</i> (July 20-23)									
Composition of nutrient solution.....			4.08	8.97	5.42	11.9	1.30		
Absorption from nutrient solution.....			3.22	1.80	.82	7.5	.36		1180
Absorption from nutrient solution plus NaCl.....	87.0	2000	.56	.70	.74	5.9	.17		810
<i>Experiment C—7-Day Period</i> (March 30—April 7)									
Composition of nutrient solution.....			4.76	9.20	3.54	11.2	1.25		
Absorption from nutrient solution.....			4.50	4.29	.74	11.0	.95		1860
Absorption from nutrient solution plus NaCl.....	87.0	2000	3.07	3.74	.99	11.0	.78		1760

\* Average of duplicate sets. Maximum difference in absorption R. V. K .3, Ca .15, Mg .24, NO<sub>3</sub> .48, PO<sub>4</sub> .06.

experiment, and the total absorption noted. It was hoped that the procedure just outlined would permit comparisons to be made of the intake of ions by similar plant systems, in which differences in growth and cumulative effects would be minimized.

The results obtained, calculated in terms of reaction values,\* are set forth in table 1. It is evident that in all the experiments a marked and consistent depression of the absorption of potassium occurred. The intake of calcium was also depressed, but not so consistently. The values for the absorption of magnesium are small, and any interpretation of the results uncertain. In two of the cases no effect was produced on the  $\text{NO}_3$  ion, and in the other case the percentage effect was much less than for several other ions. The absorption of  $\text{PO}_4$  was depressed markedly in one experiment. The main suggestion gained from these studies is that the absorption of potassium, and possibly of other ions, is depressed when a relatively high concentration of sodium salts is present in the solution. The reduction in water intake was not sufficient to account for all of the effects noted. It may be added that in other experiments similarly conducted in which calcium salts were used alone and in the presence of sodium chlorid, the absorption of calcium was depressed very significantly. When calcium was present below a certain concentration, the presence of sodium salt caused a loss of calcium from the plant, while at higher concentrations absorption of calcium occurred. The loss of calcium to the solution may, of course, be attributed to leaching from dead cells, but it is probable that a chemical displacement of calcium also occurs. In every case sodium and chlorin were removed from solution in appreciable quantities.

In continuation of these studies barley plants were grown for longer periods in culture solutions containing sodium salts. In two cases the plants were first grown in the unmodified culture solution and then transferred to solutions containing the sodium salts. In one experiment the plants were grown continuously in culture solutions plus sodium chlorid or sodium sulfate. In the solution culture experiments a large number of plants was grown in each solution. The details of these experiments and analyses of the plants obtained are given in tables 2 and 3. It is quite apparent that in every instance the presence of sodium salts in the culture solution caused a marked and significant

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\*  $\frac{\text{Valence}}{\text{atm. wt.}} \times \text{p.p.m.} = \text{milliequivalents.}$

TABLE 2.—COMPOSITION OF THE TOPS AND ROOTS OF PLANTS GROWN IN NUTRIENT SOLUTIONS, AND IN NUTRIENT SOLUTIONS CONTAINING SALTS

Description of nutrient solution	Composition of tops of plants (Air dry basis) Per cent							Composition of roots of plants (Air dry basis) Per cent							Per cent of total Cl absorbed	Total weight of tops green	Total weight of tops dry	Total weight of roots dry	No. of plants
	Salt added		N	PO <sub>4</sub>	Ca	Mg	K	Cl	N	PO <sub>4</sub>	Ca	Mg	K	Cl					
	R. V.	P. P. M.																	
Nutrient.....			3.15	1.96	1.55	.50	3.76	Experiment A	2.45	2.76	1.33	.25	1.72		920	114	26.9	35	
Nutrient plus NaCl	17.1	1000	3.10	2.06	1.22	.37	3.87		2.30	3.02	1.25	.19	1.90	4.70	68.0	1150	109	25.6	36
Nutrient plus NaCl	85.5	5000	3.06	2.20	.72	.25	2.60		7.78	2.70	3.53	.64	.17	1.96	6.20	25.0	780	140	24.5
			Experiment B																
Nutrient.....			5.24	2.42	1.23		6.11		4.48	2.26	1.32		5.06		232	25	7	140	
Nutrient plus NaCl	85.5	5000	4.42	2.58	.58		3.85		4.06	3.38	.43		2.90		177	21	7	140	

*Experiment A.*—Plants grown in nutrient solution for 1 month, then changed to solutions described above and grown 4 weeks. (September 17–October 14.)

*Experiment B.*—Plants grown 1 week in nutrient solutions, and then 4 weeks in solutions as described, with frequent changes of solution. (April 14–May 12.)

TABLE 3.—COMPOSITION OF PLANTS GROWN IN SAND CULTURES IN NUTRIENT SOLUTIONS WITH AND WITHOUT ADDITION OF SODIUM SALTS

	Description of nutrient solution		Percentage of water free material									
	Salt added		Freezing point depression of solution, degrees C.	K	Ca	Mg	Na	N	PO <sub>4</sub>	Cl	SO <sub>4</sub>	Total P
	R.V.	P.P.M.										
<i>Nutrient solution</i> .....			.057	1.52	.89	.32		.44	.26		1.08	.55
Stems and leaves.....				.47	.06	.12		1.54	1.15		.09	
Grain.....				1.31	.61	.35		.50	.38		.59	
Chaff.....												
<i>Nutrient solution + NaCl</i> .....	51.3	3000	.237	1.78	.71	.23	1.65	.47	.25	2.58		.49
Stems and leaves.....				.43	.07	.13		1.69	1.03	.17		
Grain.....				1.87	.79	.28		.55	.30	.98		
Chaff.....												
<i>Nutrient solution + NaCl</i> .....	102.6	6000	.418	2.02	.63	.21	2.58	.63	.25	3.59		.49
Stems and leaves.....				.45	.05	.10		1.97	1.02	.25		
Grain.....				2.25	.53	.27		.61	.29	1.69		
Chaff.....												
<i>Nutrient solution + Na<sub>2</sub>SO<sub>4</sub></i> .....	70.4	5000	.223	1.16	.49	.16	1.43	.46	.34		1.78	.78
Stems and leaves.....				.37	.07	.15		1.69	1.05		.14	
Grain.....				1.39	.43	.23		.54	.32		1.14	
Chaff.....												
<i>Nutrient Solution + Na<sub>2</sub>SO<sub>4</sub></i> .....	140.8	10000	.374	.90	.48	.15	2.39	.45	.34		2.21	.97
Stems and leaves.....				.55	.05	.12		1.86	1.09		.12	
Grain.....				1.73	.46	.25		.61	.34		1.70	
Chaff.....												

Period of growth, May to September.



alteration in the composition of the plant tissue. In general, the percentages of cations were depressed, while relatively smaller effects are noted in the case of the anions. In several instances the percentages of nitrogen and phosphorus were increased to a greater or less extent. In the experiment in which plants were grown to maturity in solutions (sand cultures) containing sodium chlorid, some increase occurred in the percentage of potassium found in the stems and leaves and chaff. The total quantities of potassium absorbed on the average by a plant were similar, however, for the culture solution and for the culture solution plus sodium chlorid (table 4). Where there was a decreased yield of crop, the percentage of potassium increased correspondingly. It would seem, therefore, that under these circumstances the decreased rate of absorption of potassium did not depress the total intake of potassium when the entire period of growth was considered, as a large proportion of all of the potassium present in the solution was removed. This does not apply to calcium and magnesium, since these elements are also found in smaller percentages and total quantities in the mature plants. The plants grown in the solutions to which the sodium sulfate was added differ from the plants grown in the sodium chlorid solution in that the percentages in stems and leaves and the total quantities of potassium absorbed are decreased to a marked extent, especially in the case of the higher concentration of sulfate. A greater effect was also produced on the content of calcium and magnesium. No significant decreases are noted in the percentage of nitrogen and phosphorus in any portion of the plant. Marked differences in the composition of the grain are not evident, except a possible increase in the percentage of nitrogen.

The total equivalents of sodium present in the stems and leaves exceeded those of potassium, calcium, or magnesium, when sodium was present in the culture solution. Sodium also tended to cause a change in the relation of potassium to calcium by decreasing the proportion of the latter.

Very considerable percentages of chlorin and sodium are stored in the stems and leaves, and the grain itself contains appreciable quantities of chlorin. The previous solution culture experiments showed a still greater accumulation of chlorin (and presumably of sodium) in the plant tissue. The equivalent weight of sulfate

TABLE 4.—TOTAL QUANTITIES OF INORGANIC ELEMENTS ABSORBED BY PLANTS GROWING IN SAND CULTURES WITH AND WITHOUT ADDITION OF SODIUM SALTS  
(Average per Plant)

	Description of nutrient solution			Weight of dry material Gms.	K	Ca	Mg	Na	N	PO <sub>4</sub>	Cl	SO <sub>4</sub> (in ash)	Total S (Calculated to SO <sub>4</sub> )
	Salt added		Freezing point depression of solution, degrees C.										
	R. V.	P. P. M.											
<i>Nutrient solution:</i>							(Gram	<i>equivalents x 1000</i> )					
Stems and leaves.....			2.057	6.63	2.59	2.94	1.73		2.07	.18		1.50	2.23
Grain.....				9.92	1.20	.29	.99		10.94	1.20		.10	
Chaff.....				1.50	.51	.45	.41		.57	.06		.19	
Total.....				18.05	4.30	3.68	3.13		13.58	1.44		1.79	
<i>Nutrient solution plus NaCl.</i>													
Stems and leaves.....	51.3	3000	.237	5.75	2.64	2.05	1.07	4.13	1.93	.15	4.17		1.74
Grain.....				11.63	1.28	.40	1.23		14.01	1.26	.56		
Chaff.....				1.63	.79	.65	.33		.64	.05	.45		
Total.....				19.01	4.71	3.10	2.63		16.59	1.46	5.18		
<i>Nutrient solution plus NaCl.</i>	102.6	6000	.418										
Stems and leaves.....				4.31	2.23	1.35	.74	4.83	1.93	.12	4.37		1.30
Grain.....				9.69	1.13	.25	.82		13.66	1.04	.68		
Chaff.....				1.31	.74	.35	.33		.57	.04	.62		
Total.....				15.31	4.10	1.95	1.89		16.16	1.20	5.67		
<i>Nutrient solution plus Na<sub>2</sub>SO<sub>4</sub></i>	70.4	5000	.223										
Stems and leaves.....				5.94	1.77	1.55	.74	3.70	2.00	.21		2.20	2.85
Grain.....				9.75	.92	.35	1.15		11.80	1.08		.29	
Chaff.....				1.69	.61	.40	.33		.64	.06		.40	
Total.....				17.38	3.30	2.30	2.22		14.44	1.35		2.89	
<i>Nutrient solution plus Na<sub>2</sub>SO<sub>4</sub></i>	140.8	10000	.374										
Stems and leaves.....				3.94	.92	.95	.49	4.09	1.29	.14		1.81	2.36
Grains.....				7.00	.97	.20	.66		9.29	.80		.16	
Chaff.....				.94	.41	.20	.25		.43	.03		.33	
Total.....				11.88	2.30	1.35	1.40		11.01	.97		2.30	

16 to 24 plants used for each solution.

The number of plants does not warrant statistical treatment, but the large and consistent differences in composition, as shown above, indicate that variability does not invalidate the conclusions reached.

absorbed was decidedly less than that of chlorin. This relationship is especially brought out by a comparison of the figures representing the percentages removed of the total chlorin and of the total sulfate present in the solution, this value for the chlorin being from three to seven times that for the sulfate. The equivalent weight of sodium stored in the stems and leaves considerably exceeds that of the total sulfur. These facts are in accord with the results of other experiments carried out in this laboratory, to be described later, which indicate that the sulfate ion is absorbed by barley at a relatively slow rate, while the activity of chlorin in this regard is sometimes more nearly comparable to that of the nitrate ion.

With regard to the crop production (table 4), it may be noted that slight decreases in total weight of stems and leaves were brought about by the lower concentrations of salts, with more pronounced effects for the higher concentrations. For similar osmotic concentrations sodium chlorid and sodium sulfate produced similar decreases in yield. No decrease in the grain produced is noted, except in the case of the high sulfate solution. Considering the relatively small number of plants grown in the sand cultures, the decrease of yield with the lower concentrations of salt may not be significant. No certainly significant differences were noted in respect to height, number of heads, or of tillers. In general, the salts in the concentrations used did not cause visible injury to the plant other than the decrease in yield. The plants grown in the salt solutions matured a little earlier than those grown in the unmodified nutrient solution.

It may be stated here, incidentally, that other sand and solution culture experiments with barley do not indicate that sodium chlorid is definitely more toxic than the sulfate when equal osmotic concentrations are compared. The comparison of the salts on a percentage basis according to the usual custom would probably point to the greater toxicity of sodium chlorid, but from a physiological standpoint it appears that the comparisons should rather be made on the basis of osmotic concentrations or of equal concentrations of sodium. Observations on the relative toxicity of these salts when present in the soil introduce many complicating questions concerning the actual concentrations of the various ions in the soil solution. It will be recalled that Hilgard placed the relative toxicity of sodium chlorid and sulfate as five to one.

Several experiments were also carried out with cucumbers and cantaloupes, comparing the effects of sodium chlorid and sulfate solutions of equal osmotic concentrations. With this type of plants it was also found that sodium sulfate was not less toxic than sodium chlorid and in several instances, in fact, the former salt appeared to be even more toxic to these plants. The absorption of electrolytes by the plants was greater from the chlorid than from the sulfate solution under the experimental conditions. From other considerations it is probable that the relatively smaller absorption of ions from the sulfate solution was not simply the result of inhibited plant growth, but was,

TABLE 5.—EFFECT OF SODIUM SALTS ON ABSORPTION OF CALCIUM BY BEANS

Description of solution	Salt added		Dry of tops, Grms.	Ca %	Total Ca Grms.
	R.V.	P.P.M.			
Control.....			8.2	1.84	.151
Control + NaCl.....	17.1	1000	9.0	1.58	.142
Control + Na <sub>2</sub> SO <sub>4</sub> .....	17.1	1215	9.9	1.43	.142
Control + NaCl.....	51.3	3000	5.6	1.31	.073
Control + Na <sub>2</sub> SO <sub>4</sub> .....	51.3	3645	6.0	1.22	.073
Control + NaCl.....	85.5	5000	3.4	1.20	.041
Control + Na <sub>2</sub> SO <sub>4</sub> .....	85.5	6075	4.6	1.16	.053

Plants grown for 6 weeks in sand culture. 25 plants used for each solution.

Analyses averages of closely agreeing duplicate determinations.

in part, related to the slow rate of penetration of the sulphate ion. It might be suggested here that an increased rate of absorption of ions would tend toward the attainment of osmotic relations between solution and plant which would favor the absorption of water.

One experiment was also made with bean plants (small white) in sand culture. The results presented in table 5 show very appreciable decreases in the percentages of calcium contained in the stems and leaves when the plants were grown in solutions to which sodium chlorid or sulfate was added.

The next question considered pertained to the relative toxicity and the absorption of ions with a salt of a different type. Comparison was, therefore, made between solutions containing sodium salts and others containing calcium chlorid. These experiments with barley



indicated that for equal concentrations of chlorin and similar osmotic values calcium chlorid was more toxic than sodium chlorid at the higher concentrations. Special absorption studies were also made with single salt solutions of calcium chlorid to determine the relative rate of intake of calcium and of chlorin ions. It was found that, under the conditions of the experiment, the reacting weights of chlorin absorbed exceeded those of calcium. The ionic balance in the solution was restored by the excretion or formation of  $\text{HCO}_3$  ions, the reaction of the solution becoming practically neutral. The injury to the barley plants grown during the summer months in calcium chlorid solutions containing 5000 p.p.m. of calcium chlorid or more was very marked, whether the calcium chlorid was used alone or was added to a complete nutrient solution. The leaf injury appeared to be more pronounced than the root injury, at least in the first stages. From a few preliminary tests it appeared that the toxicity of magnesium chlorid was of similar type, but more intense. Sand culture experiments with calcium chlorid gave results similar to those obtained from solution cultures.

Since the toxicity of the calcium chlorid<sup>3</sup> to barley was greater than might have been anticipated, some consideration was given to the possibility that impurities of a toxic nature were present in the salt used. Numerous tests were made for toxic elements which might conceivably have contaminated the salt during the processes of manufacture, but so far the results obtained do not indicate that the effects produced on the plant can be ascribed to this cause. It is not desired at present, however, to draw any final conclusions concerning the relative toxicity of various salts, since a systematic study of the question is now being made. Toxicity is intimately related to seasonal conditions, as will be pointed out later in the discussion. During winter the toxicity of calcium chlorid and of other salts has been found to be far less marked than during summer or spring. It should also be noted that statements concerning relative toxicities will vary according to the criteria used. Thus a study of the effect on the plant as a whole, after a sufficient exposure to the salt-containing solution, may not lead to the same conclusion as observations on root growth over a brief interval of time. This is particularly true when single salt solutions are used in determining root injury, as in the experiments of Kearney and Cameron.<sup>4</sup>

## EFFECT OF SALTS ON REACTION, BUFFER SYSTEM, AND OSMOTIC VALUES OF THE EXPRESSED PLANT SAP

Since 'alkali' salts were found to be readily absorbed by the plant, it was reasonable to suppose that certain corresponding changes might be produced in the composition or chemical constants of the expressed plant sap.\*

The following determinations were made on the plant juices in an attempt to ascertain what modifications were produced by the salts added to the culture solution: freezing point depression, hydrogen ion concentration, and buffer effect.

The technique of the culture experiments was simple, and consisted in growing a large number of plants (about 500) in shallow pans filled with a culture solution. The plants were supported on wooden frames fitting over the top of the pan and covered with wide-meshed mosquito netting lightly impregnated with paraffin. After the plants had been grown for several weeks in this way, the solutions were replaced by new supplies of culture solution to which were added the desired quantities of salts to be tested. In this way the effects of the salts on plants of similar development were compared. The period of contact with the salt solutions was comparatively brief, the intention being to produce only the first stages of injury. The plants were then removed from the solution and rinsed with distilled water; the roots and tops were separated, placed in jars, and then frozen in a refrigerator room at  $-15^{\circ}$  C. The material, after being frozen, was thawed quickly, the juice expressed with the aid of an ordinary screw press, and then rapidly filtered through paper pulp with the aid of suction. Determinations on the juices were made immediately after expression and filtration. It is well known that the technique and pressure employed affect the composition of plant juices. In these experiments the differences produced by the various treatments are frequently considerable and only comparative values are sought. It has been pointed out by Haas<sup>5</sup> that it is desirable to examine the different parts of the plants (such as stems, leaves, and petioles) separately. While this has not been done in the present work, the

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\* The sap (or juices) expressed from a plant cannot, of course, be assumed to be identical with the true cell sap.



nature of the experimental conditions renders it probable that the same relative proportions of the different parts were present in the comparable sets.

Three different types of plants were used in the studies on plant sap, barley (Beldi variety), peas (field), and pumpkins (Connecticut field). In one experiment barley and pumpkins were grown together

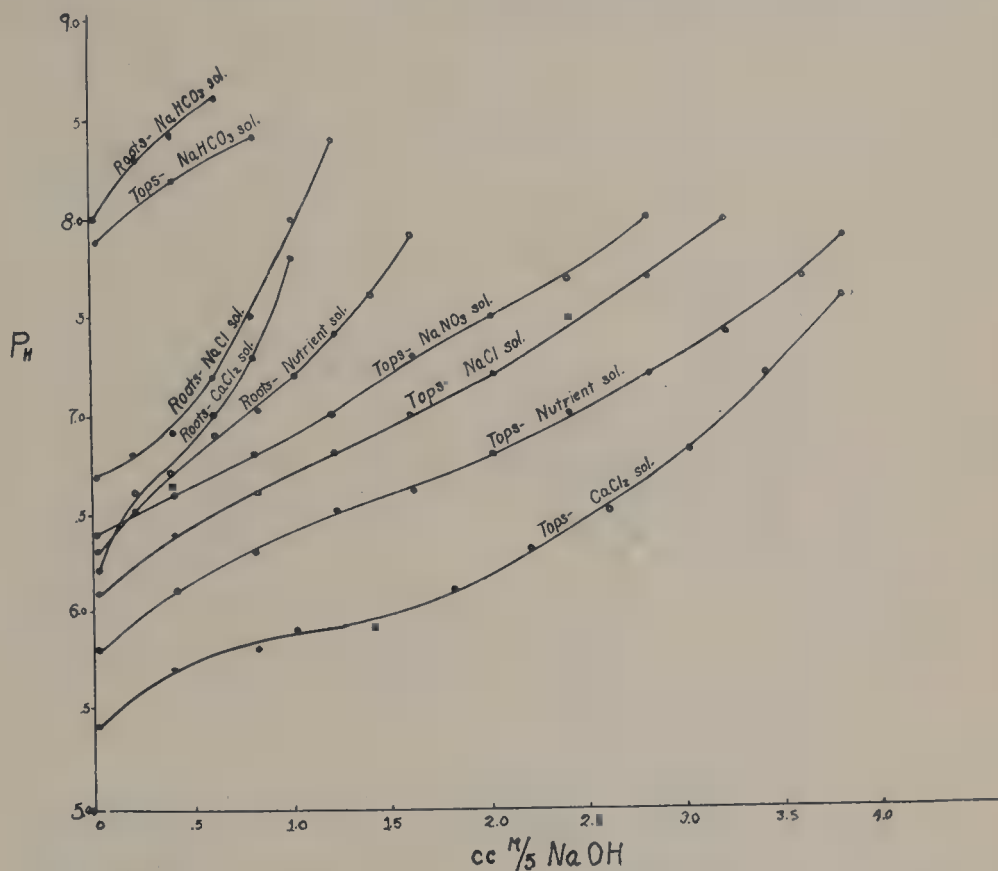


Fig. 1. Titration curves for sap (25 c.c.) expressed from barley plants grown in nutrient solution and in nutrient solutions with the addition of various salts. For details, see table 6, Experiment A.

in mixed culture, in order that the roots of both plants might always be in contact with the same solution.

Considering first the hydrogen ion concentration and titration values of the sap from the barley plants, it is observed that, in the sap expressed from the tops of the plants, the calcium chlorid solution depressed the pH value and increased the quantity of alkali required in the titration to a given pH. The sodium bicarbonate (fig. 1) brought the reaction of the sap to a distinctly alkaline point. Sodium nitrate

acted in the same direction, but to a less extent. The least change occurred in the case of the sodium chlorid solution, although a slight increase of pH and a decreased buffer effect was observed. The sap expressed from the roots grown in the sodium bicarbonate solution showed a decided increase of alkalinity and a decrease of titration value. A slightly increased pH value was produced by the sodium chlorid solution. Calcium chlorid, however, did not give rise to the same effect on the expressed root sap as it did on the sap from the tops. The change produced in the roots was small and consequently may fall within the limits of experimental error.

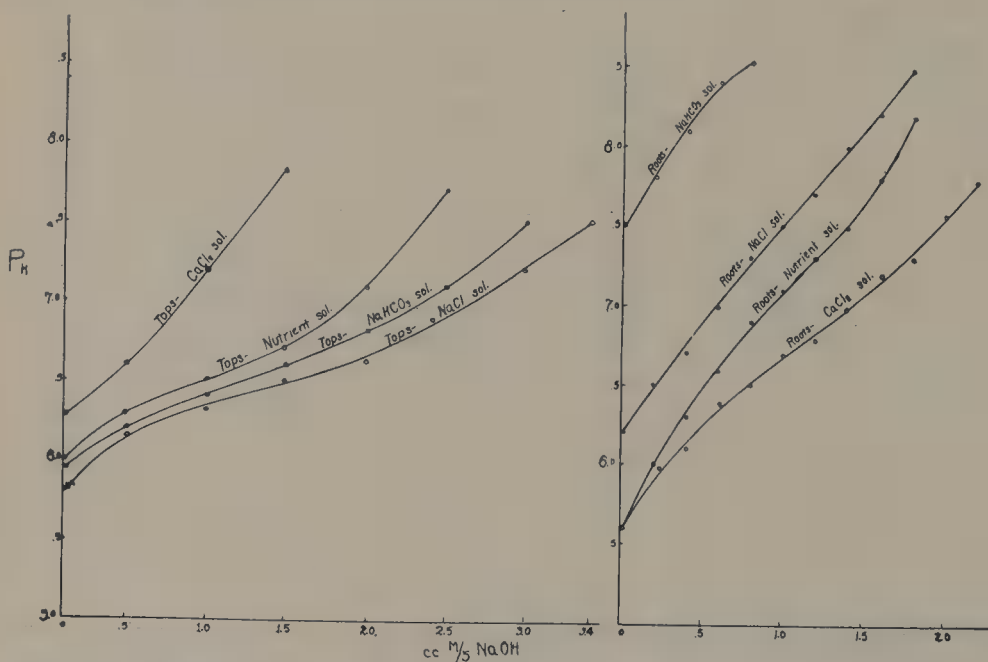


Fig. 2. Titration curves for sap (25 c.c.) expressed from pumpkin plants grown in nutrient solution, and in nutrient solutions with the addition of various salts. For details, see table 6, Experiment B.

Calcium chlorid caused the most marked injury to the plant as a whole under the given experimental conditions. The greatest injury to the roots occurred in the solutions to which sodium bicarbonate was added, but it is interesting to note that a similar, although much less extensive, injury to the roots was produced with sodium nitrate. In the solution containing sodium nitrate there was no increase of alkalinity over that of the culture solution. Nevertheless, the reaction of the expressed root sap was increased from pH 6.3 to pH 6.9, as indicated by colorimetric estimation.

The experiments with pumpkins showed, for the tops, a different set of relations in the expressed sap (fig. 2). The calcium chlorid curve for the tops was displaced to the alkaline side, instead of to the acid side as in the case of barley. Both the sodium bicarbonate and sodium chlorid curves were displaced slightly to the acid side. The root sap exhibited much the same behavior as that from barley, except that the calcium chlorid caused a definite increase in the acid reserve.

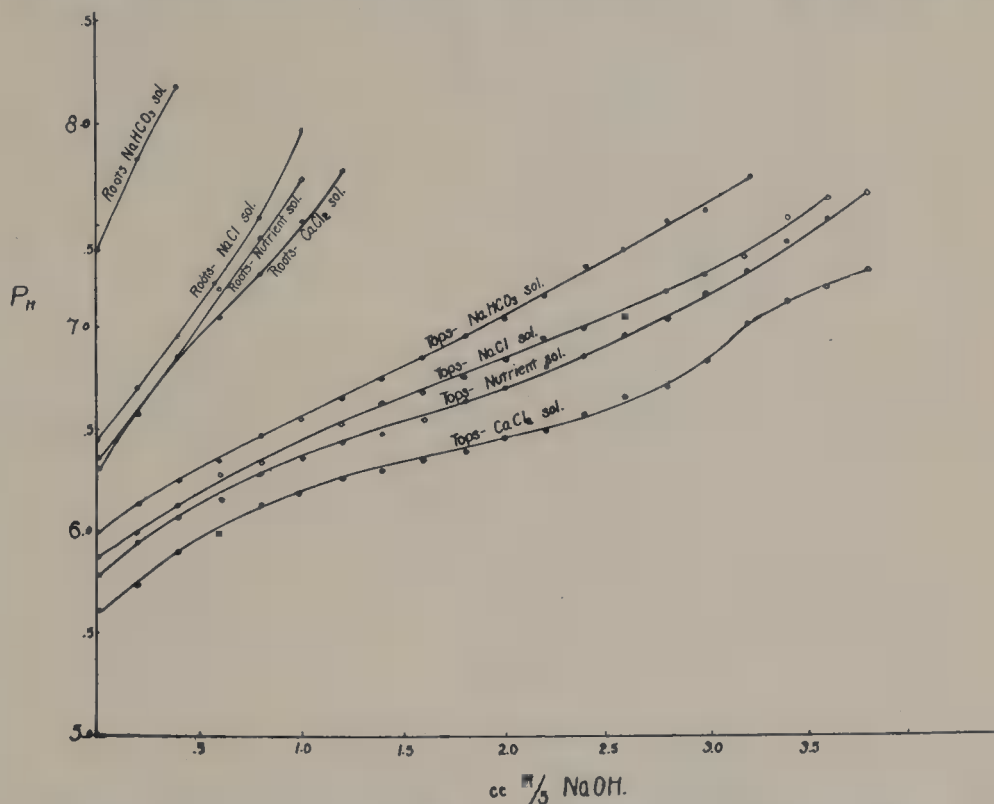


Fig. 3. Titration curves for sap (25 c.c.) expressed from barley plants grown (together with pumpkins) in nutrient solution, and in nutrient solutions with the addition of various salts. For details, see table 6, Experiment C.

Sodium bicarbonate caused the reaction to be changed from acid to alkaline, a change which was accompanied by marked injury to the roots.

Since the concentrations of salts were different in the two experiments just described, another experiment was carried out in which barley and pumpkins were grown in the same solution, each pan containing both kinds of plants, divided half and half. Smaller concentrations of salts were used (table 6). The general relations between the different curves are very similar to those obtained in the previous experiments (figs. 3 and 4).

TABLE 6.—DETAILS OF EXPERIMENTS TO WHICH PLATES 1 TO 5 REFER

*Experiment A (Barley)*

Salts added to 4 liters of nutrient solution in several portions: Total R.V.

NaCl	20 g.—10 g.—10 g.....	171
NaHCO <sub>3</sub>	12 g.— 8 g.—10 g.—10 g. ....	120
CaCl <sub>2</sub> .2H <sub>2</sub> O	31 g.—15 g.—15 g. ....	188
NaNO <sub>3</sub>	30 g.—15 g.—15 g. ....	178

Except in solution to which NaHCO<sub>3</sub> was added, the reaction was maintained as approximately neutral. Reaction in nutrient solution + NaHCO<sub>3</sub> pH 8.1—8.8.

Marked injury to whole plant with CaCl<sub>2</sub>; greatest injury to roots with NaHCO<sub>3</sub>; similar but less injury with NaNO<sub>3</sub>; slight injury to tops with NaNO<sub>3</sub>, NaHCO<sub>3</sub>, and NaCl.

All plants grown 17 days in nutrient solution and 19 days in solutions described above. (April 29—May 17.)

*Experiment B (Pumpkins)*

Salts added to 4 liters of nutrient solution: Total R.V.

NaCl	10.2 g. ....	44
NaHCO <sub>3</sub>	14.8 g. ....	44
CaCl <sub>2</sub> .2H <sub>2</sub> O	12.5 g. ....	39

Plants grown 2 weeks in nutrient solution and 12 days in solutions described above. (March 7—March 18.)

Some yellowing of plants in NaHCO<sub>3</sub> solution.

*Experiment C (Barley and Pumpkins)*

Salts added to 4 liters of nutrient solution: Total R.V.

CaCl <sub>2</sub> .2H <sub>2</sub> O	31 g. ....	95
NaHCO <sub>3</sub>	20 g. ....	60
NaCl	20 g. ....	86

pH values at end of experiment:

Nutrient solution.....	6.8
Nutrient solution plus CaCl <sub>2</sub> .....	6.8
Nutrient solution plus NaCl.....	6.8
Nutrient solution plus NaHCO <sub>3</sub> .....	8.6

Plants grown 3 weeks in nutrient solution and 1 week in solutions described above. (July 13—July 19.)

Marked injury to barley plants with CaCl<sub>2</sub>; slight injury with NaCl; injury to pumpkins in all solutions; greatest with NaHCO<sub>3</sub>. Root injury to both barley and pumpkins with NaHCO<sub>3</sub>.

*Experiment D (Peas)*

Salts added to 4 liters of nutrient solution: Total R.V.

NaCl	20 g. ....	86
CaCl <sub>2</sub> .2H <sub>2</sub> O	31 g. ....	95
NaHCO <sub>3</sub>	20 g. ....	60

Plants grown 3 weeks in nutrient solution and 1 week in solutions described above. (September 8—16.)

Appreciable injury with NaHCO<sub>3</sub> and NaCl solutions, less in CaCl<sub>2</sub> solution.

A similar experiment was also made with peas (fig. 5). No appreciable change in hydrogen ion concentration was produced in the sap expressed from the tops, although some displacement of the curves for calcium chlorid and sodium bicarbonate may be noted. The only extensive change in the root sap occurred when sodium bicarbonate was present in the solution.

It may be concluded from these examinations on plant sap that the buffer effect of the roots is in all cases definitely smaller than that

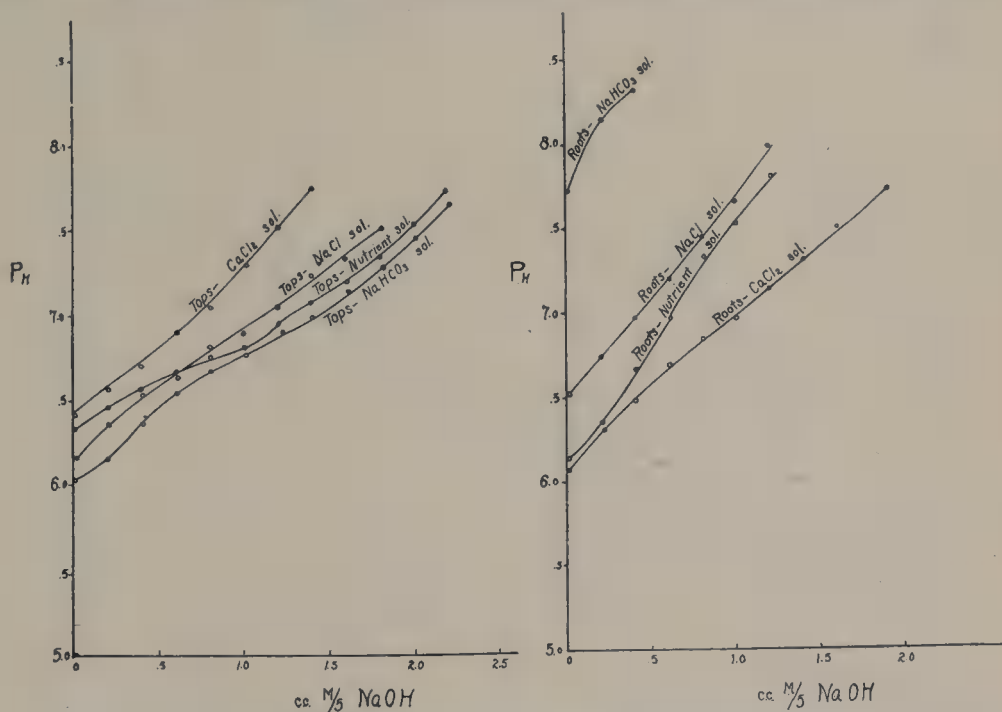


Fig. 4. Titration curves for the sap (25 c.c.) expressed from pumpkin plants grown (together with barley) in nutrient solution, and in nutrient solutions with the addition of various salts. For details, see table 6, Experiment C.

of the tops, a fact which is in agreement with the data obtained by Haas<sup>5</sup> and Kappen.<sup>6</sup> Alkaline solutions, such as are formed even with relatively moderate concentrations of sodium bicarbonate, may produce very marked changes in the reaction of the root sap, while the sap expressed from the tops is more difficult to alter, though with more prolonged treatment its reaction may likewise be changed. Other salts may also affect the reaction and buffer system of the sap, and in this respect calcium chlorid in the higher concentrations seems to exercise a significant influence, also sodium nitrate. It may be added that small increases in the pH value of the expressed sap may cause certain constituents to precipitate.

The determinations of hydrogen ion concentration and titration curves were made by means of a hydrogen electrode as described elsewhere. Such determinations do not have a high degree of accuracy, but can usually be duplicated to within .1 pH. Under the conditions of the experiments, reduction of nitrate did not affect the results, as is shown by the fact that the root sap gave similar values by both colorimetric and electrometric methods. An exception was found in the case of the roots grown in the high sodium nitrate solutions.

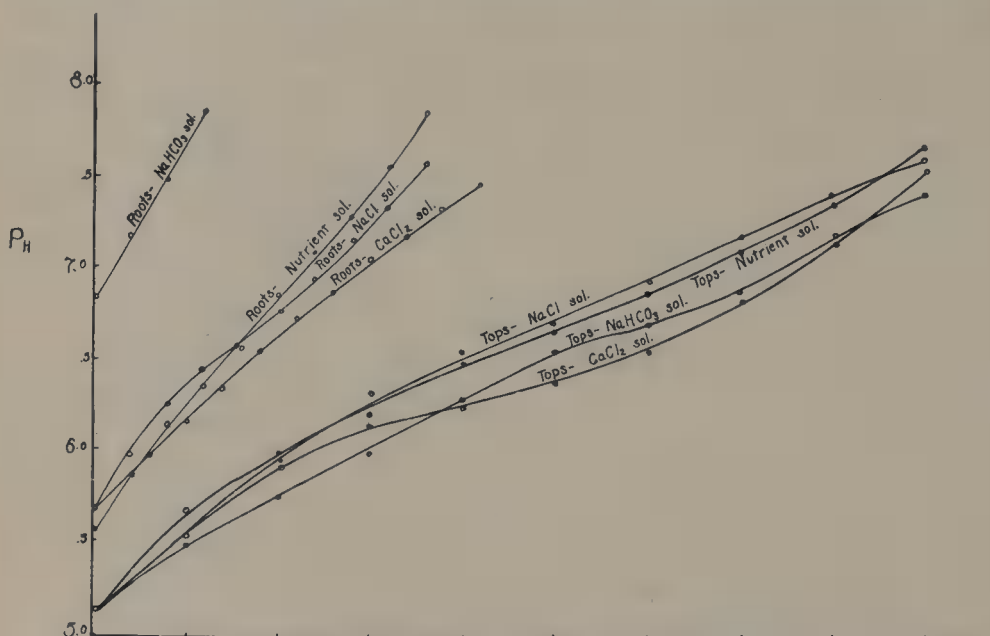


Fig. 5. Titration curves for the sap (25 c.c.) expressed from pea plants grown in nutrient solution, and in nutrient solutions with the addition of various salts. For details, see table 6, Experiment D.

At the time that the reaction of the sap was determined, observations were also made on its osmotic value as shown by freezing point depressions (table 7). It was hoped that these measurements would give some idea of the relations existing between the solution and the sap expressed from roots and tops of plants grown in solutions of various salts and exhibiting different degrees of injury. In all cases the osmotic values of the sap were increased, as was to be expected. The greatest increases were produced by sodium chlorid, which, especially in the roots, has a considerably greater effect than calcium chlorid. In several cases the root sap had a lower osmotic value than the solution in contact with the roots. The different plants attain different equilibrium points in this regard; for example, with the



TABLE 7—FREEZING POINT DEPRESSIONS OF SAP EXPRESSED FROM PLANTS GROWING IN VARIOUS SOLUTIONS

<i>Experiment A</i>			
	Solution at end of experiment °C.	Sap from tops °C.	Sap from roots °C.
Barley, nutrient solution.....	.029	.637	.325
Barley, nutrient solution plus NaCl.....	.592	1.172	.460
Barley, nutrient solution plus CaCl <sub>2</sub> .....	.462	.935	.367
Barley, nutrient solution plus NaHCO <sub>3</sub> .....	.367	1.087	.365
Barley, nutrient solution plus NaNO <sub>3</sub> .....	.612	1.137	.445
<i>Experiment C</i>			
Barley, nutrient solution.....	.007	.545	.263
Pumpkin, nutrient solution.....	.....	.437	.243
Barley, nutrient solution plus NaCl.....	.317	.902	.475
Pumpkin, nutrient solution plus NaCl.....	.....	.655	.435
Barley, nutrient solution plus CaCl <sub>2</sub> .....	.287	.602	.271
Pumpkin, nutrient solution plus CaCl <sub>2</sub> .....	.....	.567	.343
Barley, nutrient solution plus NaHCO <sub>3</sub> .....	.247	.692	.328
Pumpkin, nutrient solution plus NaHCO <sub>3</sub> .....	.....	.557	.393
<i>Experiment D</i>			
Peas, nutrient solution.....	.054	.549	.261
Peas, nutrient solution plus NaCl.....	.439	.884	.391
Peas, nutrient solution plus CaCl <sub>2</sub> .....	.400	.704	.306
Peas, nutrient solution plus NaHCO <sub>3</sub> .....	.286	.567	.376

(For details of experiments see Table 6.)

same solution barley was able to increase the osmotic pressure of the sap to a greater extent than pumpkins could in the given time. Conductivity measurements were also made on the plant juices, and these gave evidence that most of the increases of osmotic pressure may be ascribed to electrolytes.

DISCUSSION

All of the experiments described in this paper lead to the conclusion that the presence of 'alkali' salts in the nutrient solution causes marked changes in the absorption of inorganic elements by the plants under consideration. These changes may occur even when the concentration of salt is not sufficiently high to produce great injury. Alterations may likewise be brought about in the reaction and buffer systems of the tops and roots. It is open to question, however, whether

any considerable modifications in the normal reaction of the sap occur except when accompanied by definite injury. (Some recent work on this question is reported by Bauer and Haas.<sup>7</sup>) The roots are especially susceptible to such injury and change of reaction when an alkaline solution of high buffer value is used. Doubtless alterations in permeability occur and the buffer effect in the roots is not sufficiently strong to maintain the normal reaction, even when the contact with sodium bicarbonate solutions is relatively brief. In the case of a culture solution containing calcium chlorid, no unfavorable hydrogen ion concentration is present in the solution. Nevertheless, the reaction and buffer system of the sap may suffer certain modifications. Absorption studies made with barley have shown that the intake of chlorin may be considerably more rapid than that of calcium, equilibrium in the solution being maintained by the excretion or formation of bicarbonate ions. This must be equivalent to the introduction of a strong acid into the plant, with a tendency toward increased hydrogen ion concentration in the sap. But other types of plants may respond in a different manner. Thus the tendency with pumpkins was in the direction of a decrease in acidity of the sap expressed from the tops of the plants. It may be suggested that in this case certain of the organic acids present were precipitated or neutralized by the absorbed calcium. No studies have, as yet been made on the absorption of calcium and chlorin by pumpkins.

Several factors are doubtless concerned in the depression of absorption of certain inorganic ions caused by the presence of sodium salts. A large excess of sodium ions might be expected to bring about a certain displacement of other cations in any chemical compounds involved in the processes of absorption or utilization. Permeability relations may also be modified. With barley, considerable changes of this nature may occur without any evidences of marked injury. It is possible, however, that other plants might be much more susceptible to such modifications in the intake of certain important elements. Also, it may be suggested that under certain soil conditions, a high sodium content in the soil solution may be accompanied by a relatively low concentration of one or more culture elements, for example, calcium. A concentration of calcium, in itself adequate, might conceivably become inadequate when sodium was present in too high concentration. These relations would apparently

partake more of the nature of nutritional effects than of antagonism in the sense in which this term is employed by Osterhout. (See Reed and Haas.<sup>8</sup>) It is true, however, that concentrations in the plant sap are likely to be far higher than those in the culture solution, so that internally saturated surface effects are not out of the question. In certain experiments additional calcium chlorid or calcium sulfate was added to nutrient solutions containing inhibiting concentrations of sodium chlorid or sulfate, without any resulting increase of growth. The use of solutions containing very low concentrations of calcium would no doubt cause further injury. This relation between sodium salts and deficient nutrient solutions is now being studied.

The detailed discussion of the influence of hydrogen ion concentration on the absorption of culture elements is being considered in another investigation to be reported by Theron.<sup>9</sup> In this connection Breazeale<sup>10</sup> carried out a series of experiments with seedlings to show the influence of sodium salts on the absorption of nitrogen, phosphorus, and potassium from culture solutions. Calcium carbonate was present in excess and the principal effects produced on the absorption of the elements referred to above are attributed to sodium carbonate either formed by inter-reactions between the salts or added originally. Calcium and magnesium were not determined.

If we consider, from a more general point of view, the effects on a plant of a so-called alkali condition in the culture solution, or in the soil solution, we are brought to the conclusion that no simple explanation will suffice. In the first place, many diverse conditions in the soil are referred to under the general term of 'alkali.' Even when we distinguish between alkaline and saline soils, we have defined the nature of the soil solution in only the crudest manner. Data which would enable any accurate classification to be made with regard to the physiological properties of alkali soils are not available at the present time.

One of the most obvious types of injury to plants growing in a saline soil is generally ascribed to unfavorable osmotic relations existing between the plant and the solution. It cannot be questioned that interference with the water intake of a plant may cause injury and even death as a result of high concentrations of electrolytes in the soil solution. We have here, however, as modifying influences the adaptability of the plant and the nature of the atmospheric environ-

ment. From the standpoint of water relations alone, the ability of some plants to absorb and store in their sap large quantities of the alkali salts present in the soil solution may be favorable, rather than otherwise, in that the necessary readjustment of the osmotic gradient may readily take place.

Some of the most perplexing cases of malnutrition are found under conditions making it improbable that osmotic forces are primarily concerned. Thus the presence of sodium salts in the culture medium may influence the general nutrition of the plant in the manner already described. Another environment in the solution highly unfavorable to the growth of most useful plants is generally associated with a high intensity of alkalinity. In solution cultures depression of growth occurs in the case of many plants of agricultural interest (including wheat, barley, peas, alfalfa, melons, etc.) when the pH value of the solution rises much above 8. This effect is produced even when complete culture solutions are employed containing as large a concentration of calcium as can be held in solution at the alkaline reaction. Solutions of similar composition are found to be entirely favorable to growth when the reaction is made slightly acid. That a high concentration of calcium may tend, with certain plants, to prevent injury otherwise caused by excessive hydroxyl ion concentration is suggested by an interesting experiment carried out by Reed and Haas<sup>11</sup> in which walnut seedlings were grown for a week or more in continuously renewed solutions of calcium hydrate. Following out this idea, a similar experiment was carried on in this laboratory with wheat, but the plants succumbed after a very short period. It is undoubtedly true that the roots of certain plants are able to make growth in solutions of hydroxyl ion concentration entirely prohibitive of development in the case of most agricultural plants. For example, Bermuda grass will grow fairly well in highly alkaline solutions (not so well, however, as in slightly acid solutions). One reason for its ability to grow under such conditions may be related to the nature of the root structure. A microscopic examination of Bermuda grass roots shows that both the type of cell and the cell arrangement of this grass are very different from those of plants easily injured by the alkalinity of a solution. We further suggest that the roots of different plants may vary widely in their organic composition and that this fact may have some bearing on degrees of tolerance to high alkalinity. It is hoped



that investigations now being conducted will make it possible to reach more definite conclusions on this point.

When the alkalinity of the culture or soil solution is sufficiently intense, rapid disintegration of the roots of the plant grown in it is ordinarily observed. The chemical composition of root tissue is but slightly understood, but it may be suggested that pectin bodies and perhaps proteins and lipoids would be particularly subject to change in an alkaline medium. With moderate alkalinity, the injury to the plant may sometimes not become manifest until after one or two months of growth. With some plants a marked chlorosis occurs. This may, or may not, be the direct result of too high a concentration of hydroxyl ion. Nutritive disturbances must also be considered, and it is possible that the absorption or assimilation of iron or other elements is involved. Direct experiments by Theron<sup>9</sup> show that the reaction of the solution modifies the relative absorption of the ions present, the absorption of nitrate being decreased in an alkaline medium.

Haas<sup>12</sup> Hempel,<sup>13</sup> and Kappen<sup>14</sup> have carried on interesting and important investigations on the buffer system of plants, but this subject has not yet received the same detailed study which has been devoted to the buffer system of the blood. It is very probable, however, that the maintenance of a proper reaction in the living plant cell is also of great importance. Unfortunately, the juices which are expressed from the plant tissue do not represent anything so definite as the blood and do not normally display such constancy of reaction, yet it is possible that any considerable change in reaction or buffer effect of the expressed sap induced by alkali salts implies some important disturbance of metabolism and colloidal condition of the protoplasm. The evidence presented in this paper suggests that such changes of reaction may be produced in plants by certain salts, particularly in the roots. Appreciable quantities of hydrolyzable salts, such as sodium bicarbonate, may be very effective in modifying the reaction of the sap, even though the solution may not show an extremely high pH value. The reserve of hydrolyzable salt and the continued maintenance of an alkaline reaction, as well as the pH value at any given moment, are important from a physiological standpoint. Also, as stated before, a solution of approximately neutral reaction may cause some modification of reaction in the plant sap,

as is shown by the results obtained with solutions containing non-hydrolyzable sodium salts or calcium chlorid. Excess of sodium nitrate acts to a certain extent like sodium bicarbonate. This fact is explainable by the rapid utilization of the nitrate ion with the formation of an alkaline residue.

Whatever may be the exact significance of the differences of reaction, it is evident that the internal chemical system of a plant may be greatly modified under so-called alkali conditions as a result of changes induced in the relations between the absorbed ions and organic complexes synthesized by the plant.

In soils maintaining an alkaline reaction, certain elements, such as calcium, magnesium, and iron may occur in the soil solution only in very low concentrations. It may be inferred that in such a medium, most plants might find it impossible to absorb certain essential elements at a sufficiently rapid rate to permit of satisfactory growth. The plant itself, however, tends to overcome the unfavorable reaction and deficiency of solutes by its ability to excrete carbon dioxide. Whether or not the desired plant growth takes place, will depend upon the buffer effect of the soil, the nature and rate of solution of the mineral components, and of course upon physical conditions, which are not now under consideration. Specific toxic compounds, organic or inorganic, other than those discussed have been suggested as contributing causes of injury, but no extensive data dealing with this phase of the question are available.

Any discussion of the effects of salts on plants would be incomplete without reference to the part played by climatic influences. The same solution may vary in toxicity to a very great extent according to the nature of the aerial environment, temperature, sunlight, and humidity. Absorption and transpiration of water and the intake of the ions of alkali salts and of the nutrient elements, may all undergo significant modification when climatic conditions are varied. It is not to be expected, therefore, that any sharp line of demarcation can be drawn between toxic and non-toxic solutions, any more than between good and poor nutrient solutions. Lipman and Davis<sup>15</sup> have shown, experimentally, that various concentrations of sodium chlorid may stimulate growth under certain conditions, while other experiments, conducted at a different time of year, gave evidence that similar solutions produced marked inhibition of growth under the new environment.



## SUMMARY

1. Sodium chlorid and sodium sulfate, when added to a culture solution, caused certain marked alterations in the absorption of inorganic elements and in the composition of the barley plant. The cations were particularly involved, the sodium salts tending to decrease the absorption of calcium, magnesium, and potassium.

2. When sodium chlorid is used, sodium and chlorin may be absorbed and stored by the barley plant in relatively large quantities. In the case of sodium sulfate, the sulfate ion is removed from solution less rapidly than the chlorin ion. This is also true of other plants than barley, experiments with cucumbers and cantaloupes having given similar results.

3. On the basis of preliminary experiments, the question is raised whether sodium chlorid possesses greater toxicity than sodium sulfate for common agricultural plants, when equal osmotic values, or equal concentrations of sodium are compared. It is concluded that no definite alkali tolerances for different plants can be established, because of the very important modifying influences of climate and season and other environmental conditions.

4. Observations were made on the effect of salts on the reaction and buffer systems of barley, peas, and pumpkins. Rapid and extreme changes in the reaction of the sap expressed from the roots were caused by the addition of sodium bicarbonate to the culture solution. The buffer effect of the sap expressed from the stems and leaves was greater than that of the sap expressed from the roots, and less subject to change of reaction. Calcium chlorid produced appreciable changes in the reaction and buffer effect of the plant juices. Barley and pumpkins were influenced in opposite directions. Neutral sodium salts also caused slight changes in reaction and titration values. Sodium nitrate in the concentration employed increased the alkalinity of the expressed root sap, with accompanying injury similar to, although less extensive than that induced by sodium bicarbonate.

5. A brief general discussion of certain phases of alkali injury to plants is given.

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EXPERIMENTS ON THE RECLAMATION OF  
ALKALI SOILS BY LEACHING WITH  
WATER AND GYPSUM

BY  
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P. L. HIBBARD

(Contribution from the Division of Plant Nutrition, California Agricultural Experiment Station)

In connection with an investigation of the effects of drainage and flooding on certain alkali soils found on the Kearney Vineyard Ranch of the University of California, one-ton lots of five different types of these soils were brought to the laboratories at Berkeley and have been given intensive study for a period of several years. The results of various pot experiments on these soils have already been reported elsewhere.<sup>1</sup> These observations indicated that it would be necessary to remove most of the alkali before successful crops could be grown. The present discussion is especially concerned with the detailed chemical examination of columns of soil which had been subjected to leaching and to gypsum treatments. In view of the importance of the alkali problems which are suggested by the study of these soils, and because of the intensive experiments in the field now being conducted by Kelley and Thomas, it seems desirable to place on record certain of the data obtained in this laboratory.

Complete analyses and descriptions of the soils were given in the article mentioned.<sup>1</sup> In general, they are classified as Madera fine sandy loam. Numbers 16, 17, 19, and 20 are very similar physically. No. 18 is of finer texture and contains more clay.

Chemically, these soils vary chiefly in their content of easily soluble salts.

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<sup>1</sup> Soil Science, vol. 13, p. 125 (Feb., 1922).



No. 16 is so highly alkaline and saline that no vegetation grows upon it.

No. 17 is similar, but has been leached in the field by flooding so that it now contains less than one-fourth as much soluble matter as 16. It also is without vegetation.

No. 18 is neutral in reaction, but so high in salt content that little will grow on it.

No. 19 contains moderate amounts of both alkaline and neutral salts. Alkali-tolerant crops grow on this soil.

No. 20 has much more alkaline and neutral salts than 19, but less than 17. Few plants grow well in it.

Table 1 shows the total amount of water-soluble alkaline and neutral salts in these soils; and the column of table 2 headed "Before Leaching," shows the amount of each of the principal ions in the original soils.

TABLE 1  
CHANGES IN ALKALI AND SALTS BY FIRST LEACHING

Soil No.:	16	17	18	19	20
Weight of original soil, in pounds.....	105	102	97	102	104
Total depth of water added; in feet.....	1.5	1.5	1.5	1.5	1.5
Time after adding water before first drip; in days.....	7	1	4	3	2
Total time of leaching; in days.....	80	72	63	68	67
Approximate per cent of alkalinity as $\text{Na}_2\text{CO}_3$ at start.....	1.06	0.32	0	0.09	0.16
Approximate per cent of alkalinity as $\text{Na}_2\text{CO}_3$ at end.....	0.42	0.13	0	0.03	0.08
Approximate per cent of salts at start.....	1.61	0.30	0.82	0.14	0.33
Approximate per cent of salts at end.....	0.20	0.05	0.16	0.04	0.03

Experiments described elsewhere (see note 2) showed that addition of gypsum and other amendments did not overcome the toxicity of these soils sufficiently to permit the production of satisfactory crops. It was therefore decided to see what could be accomplished by leaching. The new experiments were designed to determine quantitatively, as nearly as possible on a laboratory scale, the changes taking place in these soils when leached with water containing gypsum in solution. The chief points studied were:

1. Rate of leaching, or time required to free the soil of excessive alkaline and neutral salts.



2. Amount of water necessary to accomplish this leaching.
3. Quantity of soluble matter removed.
4. Depth of soil from which this soluble matter was removed by a limited amount of water.

TABLE 2

QUANTITY AND LOCATION OF THE CHIEF WATER-SOLUBLE ANIONS IN THE SOILS  
BEFORE AND AFTER THE FIRST LEACHING

Parts per million in dry soil

Soil 16					
	Before leaching	After leaching			
		Top	1 ft.	2 ft.	3 ft.
pH.....	9+	8	9+	9+	9+
Ca.....	0	300	0	0	0
CO <sub>3</sub> .....	3300	72	240	648	888
HCO <sub>3</sub> .....	3050	342	488	512	634
SO <sub>4</sub> .....	2000	2000	1000	1000	1000
Cl.....	7600	100	100	140	200
Soil 17					
pH.....	9+	8	9	9+	9+
Ca.....	0	100	0	0	0
CO <sub>3</sub> .....	600	48	168	528	720
HCO <sub>3</sub> .....	610	342	512	512	488
SO <sub>4</sub> .....	200	400	1000	1000	1000
Cl.....	1850	80	100	120	160
Soil 18					
pH.....	7	7.5	8	8	8
Ca.....	300	0	0	0	0
CO <sub>3</sub> .....	0	0	48	72	48
HCO <sub>3</sub> .....	61	340	560	537	340
SO <sub>4</sub> .....	1250	0	0	0	0
Cl.....	1275	0	0	100	260
Soil 19					
pH.....	8.2	7.2	8	8.5	9
Ca.....	0	0	0	0	0
CO <sub>3</sub> .....	48	48	96	192	240
HCO <sub>3</sub> .....	238	410	537	586	732
SO <sub>4</sub> .....	500	0	0	0	0
Cl.....	635	0	0	60	140
Soil 20					
pH.....	8.5	8	8.5	9	9
Ca.....	0	0	0	0	0
CO <sub>3</sub> .....	120	48	120	240	384
HCO <sub>3</sub> .....	183	390	439	439	268
SO <sub>4</sub> .....	500	0	0	0	0
Cl.....	1825	0	0	0	0

5. Possibility of return of the remaining soluble matter toward the surface when it had not all been removed from the lower layers of the soils.

6. Suitability of the leached soil for the growth of crops.

The soils were placed in iron sewer pipes about 6 inches in diameter and 5 feet long, tarred inside and outside. These pipes were supported in a vertical position with the bell end up, the lower end hanging free. To retain the soil, the lower end was covered with muslin supported by a strong wire gauze tied to the end of the pipe. In filling the pipes, a small amount of the dry soil was moistened and packed into the bottom of the pipe. Then the dry soil, together with some water, was added, with stirring and packing, until the pipe was full. This procedure was followed in order to avoid the considerable contraction in volume which the soil would otherwise have suffered when covered with water. In this way the pipes remained nearly full after the leaching was completed.

Gypsum was added to only the top foot of the soil, in the amount calculated to be necessary for changing the sodium carbonate in this portion of the soil to sodium sulfate. After filling with soil, the small space at the top of the pipes was kept covered, 1 to 2 inches deep, with water containing  $\frac{1}{10}$  per cent of gypsum until 8250 c.c. of water had been added to each. This quantity is equivalent to a depth of  $1\frac{1}{2}$  feet of water, probably more than would ordinarily be applied at one time in field flooding.

Percolation was comparatively rapid at first, until most of the salts had been removed, after which the rate decreased gradually. After two months, percolation became very slow. The most rapid flow was always through soil 18. This soil is finer in texture and contains more clay than any of the others; but it contains only neutral salts and no  $\text{Na}_2\text{CO}_3$ , so that it was less deflocculated by the leaching than were the others. The remaining four soils were physically similar, and percolation through them was approximately inversely proportional to their content of  $\text{Na}_2\text{CO}_3$ . Soil 16 became very nearly impervious after two months' leaching. In table 1 are summarized some of the data in respect to time and effectiveness of leaching, and the approximate alkalinity and salinity of the soils before and after leaching with  $1\frac{1}{2}$  feet of water, which is less than one-third of the

volume of the soil treated. This water removed from 70 to 90 per cent of the neutral salts in the soil, but only one-fourth to one-half of the total alkaline salts.

#### LOCATION OF THE REMAINING ALKALI AFTER THE FIRST LEACHING

After the soils had dried out sufficiently, analyses were made of samples taken from the top and from the first, the second, and the third foot. The results are given in table 2. The composition of the unleached soil is given in the first column. The analytical methods used were only approximate, but all samples were tested by the same methods, so that the results are comparable. When  $\text{SO}_4$  and Ca appear in the top soil, it is assumed that they have been derived from unchanged gypsum which was added to the top foot of soil before leaching. This is most apparent in soils 16 and 17, to which large amounts of gypsum were added.

It appears that, in every case, nearly all the chlorid was removed to below the three-foot level in the soil. Most of the  $\text{SO}_4$  also was washed down below three feet. Carbonate ( $\text{CO}_3$ ) was nearly all removed from the top, but much remained in the lower portions of the soil. Soil 18 had apparently gained in concentration of hydroxyl ions by the leaching. This effect is commonly produced by leaching the sodium salts from a neutral soil.<sup>2</sup> In the third foot of soil 18, the carbonate and bicarbonate are less than in the second foot, but here a considerable portion of the original neutral salts remains and depresses the alkalinity. It is well known that the addition of neutral salts to a slightly alkaline solution lowers the pH of the solution.<sup>3</sup>

Seemingly, the top soil in each column had been sufficiently cleared of alkali by the leaching to permit the growth of a crop. Accordingly, barley was planted in each on July 15, while the soil was still very wet. While the number of plants grown was too small to justify other than very general conclusions, it was apparent that while the soils were no longer toxic, they were, nevertheless, incapable of producing normal plants, probably because of a lack of available nutrients. In another series of experiments, it was found that soil 18 *after leaching* produced nearly as good a crop of barley as an otherwise similar soil which had never contained a high concentration of salts.

<sup>2</sup> Cf. Cummins and Kelley, Univ. Calif. Agri. Exp. Sta. Tech. Paper No. 3.

<sup>3</sup> See Clarke, *Determination of Hydrogen Ions*, p. 84.

## RISE OF ALKALI DURING GROWTH OF BARLEY

One of the chief points to be determined in this investigation was whether there would be a sufficient return of alkali to the surface, from the lower part of the soil, to produce a toxic concentration in the zone of plant roots. To give the greatest possible opportunity for a rise of the alkali, the lower ends of the soil columns were kept in jars of distilled water. After the plants were well started, no water was applied to the surface of the soil; consequently the plants had to draw their moisture from the lower part of the columns which still contained alkali. On account of the small growth of the barley, there was no great loss of water by transpiration. There was considerable evaporation from the surface of the soil, however, so that there was some rise of salts. After removal of the crop, the soils were again sampled and analyzed in the same manner as after the first leaching. It was so difficult to obtain the samples that it cannot be assumed that they were taken from exactly the same locality in the soil in both cases. They show fairly well, however, what changes had taken place during the growth of the crop. The results are given in table 3.

It appears that alkalinity, as measured by  $\text{CO}_3$  and  $\text{HCO}_3$ , had decreased in nearly all cases. But salinity, as measured by  $\text{Cl}$ , had returned toward the tops of the soil columns. Though the amount of variation is not very great, it is enough to show the tendency of the salts to rise with the capillary soil moisture. Before it can be said that an alkali soil has been reclaimed by flooding and drainage, it is evident that soluble matter will have to be removed by leaching to such a depth that the ordinary movements of soil moisture will not again bring it up to the region of plant roots.

To explain the lessened alkalinity after the growth of the first crop, the following hypothesis is offered: At the time of planting the newly leached soils were full of water and consequently contained very little air, but there was considerable gypsum in the upper foot. During the growth of the crop, the soils were well aerated, and some  $\text{CO}_2$  was released by the plant roots. Also, the free oxygen helped to form more  $\text{CO}_2$  by oxidizing organic matter in the soil. The increased  $\text{CO}_2$  changed  $\text{Na}_2\text{CO}_3$  to  $\text{NaHCO}_3$ , and the  $\text{CaSO}_4$  changed  $\text{NaHCO}_3$  to  $\text{Na}_2\text{SO}_4$ , so that the alkalinity was reduced.

THE SECOND LEACHING

After the crop of barley was harvested, leaching was again started in order to remove the residual soluble salts in the lower parts of the soil columns. The soils were kept covered most of the time with water saturated with gypsum. This treatment was continued from Novem-

TABLE 3  
QUANTITY AND LOCATION OF THE CHIEF WATER-SOLUBLE ANIONS IN THE SOILS  
AFTER GROWTH OF FIRST CROP  
Parts per million in dry soil

Soil 16				
	Top	1 ft.	2 ft.	3 ft.
pH.....	8	8.5	9.0	9.5
Ca.....	1200	50	25	0
CO <sub>3</sub> .....	0	30	360	480
HCO <sub>3</sub> .....	183	427	670	427
SO <sub>4</sub> .....	1700	250	0	0
Cl.....	30	30	175	200
Soil 17				
pH.....	8	8.5	9.0	9.5
Ca.....	300	100	25	50
CO <sub>3</sub> .....	0	60	240	360
HCO <sub>3</sub> .....	150	458	610	793
SO <sub>4</sub> .....	600	0	100	0
Cl.....	20	25	225	350
Soil 18				
pH.....	7.5	8.0	8.0	7.5
Ca.....	50	25	25	150
CO <sub>3</sub> .....	0	0	0	0
HCO <sub>3</sub> .....	122	213	183	61
SO <sub>4</sub> .....	0	100	1000	250
Cl.....	20	50	390	585
Soil 19				
pH.....	8.5	8.5	8.5	8.5
Ca.....	100	0	0	0
CO <sub>3</sub> .....	0	30	42	48
HCO <sub>3</sub> .....	183	305	341	390
SO <sub>4</sub> .....	1000	500	500	500
Cl.....	110	225	335	350
Soil 20				
pH.....	8.0	8.5	9.0	9.0
Ca.....	100	0	0	0
CO <sub>3</sub> .....	30	120	180	180
HCO <sub>3</sub> .....	275	305	366	335
SO <sub>4</sub> .....	1500	0	250	500
Cl.....	25	30	75	250



ber 1, 1920, to May, 1921. Percolation was slow through all of them except soil 18, which was soon nearly free of saline matter. The rate of flow was very slow through soils 16 and 17, which were still very alkaline. On May 4, the percolates were tested and found nearly free of  $\text{CO}_3$ ,  $\text{SO}_4$ , and  $\text{Cl}$ , except that from soil 16, which still contained some  $\text{CO}_3$ ,  $\text{HCO}_3$ , and  $\text{Cl}$ . At this time, about a three-inch depth of the solution of gypsum was applied to each. This solution disappeared from the surfaces of soils 18, 19, and 20 in a few days, from soil 17 in 12 days, and from soil 16 in 20 days. The final percolates, except from soil 16, contained very little soluble matter other than gypsum, which was plentiful in all but those from soil 16 and soil 17. These percolates contained no calcium, but much  $\text{SO}_4$  and a little  $\text{CO}_3$ ,  $\text{HCO}_3$ , and  $\text{Cl}$ . It was considered that soils 18, 19, and 20 had been cleared of easily soluble sodium salts, while some of these still remained in 17, and considerable remained in 16.

Further attempts were then made to grow plants in the leached soils. Barley, cucumbers (apparently very sensitive to salinity and alkalinity), and peas were grown. The same conclusion was reached as before, namely that toxic concentrations of salts were probably absent, but that lack of essential plant nutrients prevented satisfactory plant growth. In following out this idea, complete analyses of water extracts of the soils were made. The results are given in table 4. The extracts were made with carbon dioxide-free-water, in a ratio of 5 parts of water to 1 of soil, and were filtered through Pasteur-Chamberland filters. The soil for these analyses was taken from the top foot only of the soil columns.

These analyses indicate that there is not enough salinity or alkalinity in the top foot of the soil to be injurious to any ordinary plant. Considerable  $\text{CaSO}_4$  is present, but probably not in injurious amounts. Potassium and phosphate are not abundant, but probably are present in quantities sufficient for much more growth than was obtained. The amount of nitrate is very low, and the total amount of nitrogen in the soils is also very low. These deficiencies are thought to partly account for the poor growth.



## TIME REQUIRED, GYPSUM USED, AND EFFECTIVENESS OF LEACHING

Table 1 summarized these points for the first leaching. The results as a whole will now be considered.

*Time.*—The first leaching lasted for 63 to 80 days, the second, 6 months. During these periods, the soils were kept covered with water. The removal of salts from soil 18 was completed in considerably less time, but some neutral salts and considerable alkalinity

TABLE 4

ANALYSIS OF WATER EXTRACTS OF LEACHED ALKALI SOILS, DECEMBER, 1921

	Parts per million in dry soil				
Soil No.:	16	17	18	19	20
Total solids.....	2750	1925	1025	975	1015
Loss on ignition.....	275	225	100	125	165
Soluble SiO <sub>2</sub> .....	68	50	82	50	50
Fe.....	0.75	0.25	0.25	1.50	0.60
Ca.....	618	342	172	207	190
Mg.....	22	22	22	18	16
Na.....	103	121	46	60	54
K.....	21	66	43	42	56
CO <sub>3</sub> .....	0	0	0	0	0
HCO <sub>3</sub> .....	153	92	140	140	140
SO <sub>4</sub> .....	1156	1075	458	515	473
Cl.....	30	15	10	15	10
NO <sub>3</sub> .....	2	8	1	8	2
PO <sub>4</sub> .....	5.50	3.70	8.70	7.50	5.20
Total N per cent.....	0.020	0.019	0.052	0.031	0.023

still remained in soil 16, and a little in soil 17. It may be inferred that complete removal of large amounts of soluble matter from soil in the field would require many months of leaching.<sup>4</sup> Observations not here recorded indicate that the time required may be lessened by allowing the soil to dry out and to aerate after a period of leaching. This increases the rate of percolation when leaching is resumed. Emphasis should also be laid on the fact that the rate of percolation in the field may be far more rapid than in columns of soil, such as those employed in this investigation.

<sup>4</sup> See article by Cameron and Patten, Jour. Am. Chem. Soc., vol. 28, p. 1639 (1906).

TABLE 5  
GYPSUM AND WATER USED IN LEACHING THE SOILS

Soil No.:	16	17	18	19	20
Dry gypsum added before leaching; in grams.....	141	44	4.42	13.1	22.5
Gypsum added in the leaching water; in grams.....	46.69	46.69	78.15	46.69	50.19
Total gypsum added; in grams.....	187.69	90.69	82.57	59.79	72.69
Total gypsum added; in pounds.....	0.231	0.112	0.102	0.074	0.089
Equivalent to tons of gypsum per acre.....	42.5	20.5	19.0	13.5	16.5
Total water used in leaching; in liters.....	32.75	32.75	45.25	31.75	34.75
Equivalent to feet in depth.....	6	6	7.7	5.8	6.3

*Gypsum Used.*—In table 5 are recorded the amounts of gypsum and of water used at various times. With the exception of soil 16 the greater part of the gypsum was added in solution. Soil 16 contained nearly 1 per cent of sodium carbonate, to neutralize which an application of gypsum equal to 32 tons an acre in the top foot was made at the start. The analyses of the leached soils given in table 4 indicate that much of the dry gypsum added at the beginning was never brought into solution. The first leaching was made with water less than half saturated with gypsum, so that it probably dissolved some gypsum from that in the soil. This was probably not the case in the second leaching, since the water in that instance was saturated with gypsum. Very large amounts of gypsum were used, amounts which would not be economically practical in the field. Much of this excess was used to increase the rate of percolation. In field practice, probably much less would be necessary. Nevertheless, as is shown in table 4, all these soils still contain considerable water-soluble sodium, presumably more or less replaceable by calcium. During the leaching the percolates were tested from time to time to find out when the sodium salts were all removed. At the end of the last leaching, the percolate from soil 18 contained much calcium; that from soil 19, a little calcium; that from soil 20, a trace; while in the percolates from soils 16 and 17 no calcium was found. These two soils were still exchanging sodium for the calcium of the gypsum. The upper part of the soil columns contained an excess of gypsum, but the amount of water passing through was not sufficient to carry

the gypsum into the lower layers where it could react with  $\text{Na}_2\text{CO}_3$  so the lower part of the soil was still alkaline. It seems scarcely necessary to point out that gypsum, present in the soil but not dissolved in the soil water, is of no use in overcoming alkalinity. It is less obvious that the deflocculation associated with the alkalinity of such soils decreases percolation so greatly that the real problem is to get enough water through the soil for the solution of an amount of gypsum sufficient to react with all the sodium carbonate. This appears to be one of the chief reasons why so much time is required to remove the alkali from the soils. For example, soil 16 received 32,750 c.c. of water, which would dissolve about 72 grams of gypsum. The calculated amount of gypsum necessary to react with all the  $\text{Na}_2\text{CO}_3$  in this soil is 705 grams. Little more than 10 per cent of this amount could be dissolved in the water applied.

TABLE 6

PER CENT OF VARIOUS IONS REMOVED BY LEACHING, STATED AS PER CENT OF THE AMOUNT ORIGINALLY PRESENT, AS DETERMINED IN A 1:5 WATER EXTRACT

Soil No.:	16	17	18	19	20
Sodium (Na).....	98	114	98	123	117
Carbonate ( $\text{CO}_3$ ).....	85	67	0	60	50
Bicarbonate ( $\text{HCO}_3$ ).....	61	70	138	59	126
Sulfate ( $\text{SO}_4$ ).....	177	183	190	181	211
Chlorid (Cl).....	87	93	79	103	102
Total solids.....	96	114	88	116	118

*Effectiveness of Leaching.*—In table 6 are given the data showing the per cent of various ions originally present which was removed by the leaching. There is much difference between the percentages of the various ions removed. Carbonate ( $\text{CO}_3$ ) and bicarbonate ( $\text{HCO}_3$ ) were least thoroughly removed; chlorid, most completely. More than 100 per cent of sulfate ( $\text{SO}_4$ ) originally present in the soil was found in the percolates, but much of this was derived from the added gypsum. Very little of the water-soluble sodium originally present remained in the soils. Some soils lost over 100 per cent of the original water-soluble sodium. This is thought to indicate that there was considerable exchange of calcium for sodium in the relatively insoluble minerals of the soil. This change may be regarded as a distinct improvement in the soils. In general, these figures accord very well with the analyses of the final percolates obtained from the soils.

The last percolates, with the exception of those from soil 18, contained considerable carbonate and chlorid, and much bicarbonate, showing that, although the tops of the columns of soils were freed of salts and alkalinity, some still remained in the lower portions.

TABLE 7

CHANGES IN WATER-SOLUBLE CALCIUM AND SULFATE PRODUCED BY LEACHING  
ALKALI SOILS WITH GYPSUM

<i>Calcium (Ca)</i>	<i>Soil No.:</i>	<i>16</i>	<i>17</i>	<i>18</i>	<i>19</i>	<i>20</i>
Added as gypsum; in lbs....		0.0954	0.0448	0.0419	0.0304	0.0366
Found in percolate; in lbs..		0.0002	0.0005	0.0131	0.0002	0.0005
Net increase in soil; in lbs.		0.0952	0.0443	0.0288	0.0302	0.0361
Net increase in soil; in per cent.....		0.0910	0.0440	0.0290	0.0300	0.0360
<i>Sulfate (SO<sub>4</sub>)</i>						
In original soil; in lbs.....		0.1880	0.0840	0.0660	0.0520	0.0570
Added in gypsum; in lbs. ..		0.2310	0.1120	0.1020	0.0740	0.0890
Total; in lbs.....		0.4190	0.1960	0.1680	0.1260	0.1460
Found in percolate; in lbs.		0.3330	0.1540	0.1240	0.0940	0.1200
Remaining in soil; in lbs.....		0.0860	0.0420	0.0440	0.0320	0.0260
Equal to per cent of SO <sub>4</sub> of original, total.....		46	50	66	61	48
Remaining SO <sub>4</sub> ; per cent in soil.....		0.0820	0.0420	0.0460	0.0320	0.0260
<i>Sodium (Na)</i>						
Per cent in original soil.....		0.9680	0.2050	0.1550	0.0710	0.1670
Per cent of original sodium which has been replaced by calcium.....		9	22	29	40	19

CHANGES IN THE CALCIUM AND SULFATE OF THE SOILS, PRODUCED BY  
LEACHING WITH GYPSUM

Table 7 shows that about half or more of the SO<sub>4</sub> in the original soil has been removed by the leaching. The remaining SO<sub>4</sub> is probably mostly present as CaSO<sub>4</sub>. Since less calcium was found in the percolates than was added as gypsum, it is inferred that the differences may be ascribed to the replacement of sodium with the formation of calcium carbonate or silicates. The sodium thus replaced by calcium would amount to from 9 to 40 per cent of the total originally present in the soils. In soil 16, which had the most sodium, only 9 per cent was replaced by calcium, although 98 per cent of the total water-soluble sodium originally present had been removed. Soil 19,

which had the lowest concentration at first, has had 40 per cent of its sodium replaced by calcium. These statements again make evident the reason why it is so difficult to leach a soil which contains much sodium carbonate, namely, that it is impossible to get a sufficient concentration of gypsum to any point in the soil to neutralize all of the sodium carbonate at that point. The alkalinity must be gradually removed, partly by leaching, and later partly by reaction with gypsum. An electrolyte of much greater solubility, such as  $\text{MgSO}_4$ , or  $\text{CaCl}_2$ , speeds up the percolation greatly.

### CONCLUSIONS

The following conclusions were reached from observations made on the leaching of five-foot columns of five different alkali soils from Kearney Vineyard:

1. Removal of all but negligible amounts of alkaline salts from the first six feet or more of a heavily impregnated soil of the type discussed in this article will require many months of leaching.

2. Soluble matter not carried below the six-foot level by leaching will return toward the surface with the capillary water when the capillary water moves upward.

3. Most of the soluble matter may be leached out by water alone. But gypsum is valuable as a flocculent to increase the rate of leaching.

4. The anions were leached out in the following order: chlorid, nitrate, sulfate, carbonate, bicarbonate.

5. Leaching removes desirable plant food as well as undesirable salts, so that a soil which has been leached long is liable to be very unproductive for some years, or until available nutrients have been accumulated again by suitable agricultural practice.

6. Removal of more than one-half of one per cent of  $\text{Na}_2\text{CO}_3$  from a soil is very slow, because of the high degree of deflocculation produced by the alkalinity and because such a concentration of alkaline salts is much greater than can be neutralized by the gypsum in an equal volume of a saturated solution of gypsum.

7. Rates of percolation through soils may be much more rapid in the field than in constricted columns of soil. Cognizance of this difference must be taken in the application of results of laboratory experiments.

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TO THE HYGROSCOPIC COEFFICIENT

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5. Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.
6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Meyer J. Heppner. June, 1923.
7. A Study of the Darkening of Apple Tissue, by E. L. Overholser and W. V. Cruess. June, 1923.
8. Effect of Salts on the Intake of Inorganic Elements and on the Buffer System of the Plant, by D. R. Hoagland and J. C. Martin. July, 1923.
9. Experiments on the Reclamation of Alkali Soils by Leaching with Water and Gypsum, by P. L. Hibbard. August, 1923.
10. The Seasonal Variation of the Soil Moisture in a Walnut Grove in Relation to Hygroscopic Coefficient, by L. D. Batchelor and H. S. Reed. September, 1923.
11. Studies on the Effects of Sodium, Potassium, and Calcium on Young Orange Trees, by H. S. Reed and A. R. C. Haas. October, 1923.

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THE SEASONAL VARIATION OF THE SOIL  
MOISTURE IN A WALNUT GROVE IN  
RELATION TO THE HYGROSCOPIC  
COEFFICIENT\*

BY

L. D. BATCHELOR AND H. S. REED†

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INTRODUCTION

The present investigation was undertaken for the purpose of studying seasonal changes in the soil moisture and their relationship to certain phases of the activity of the trees. The work to be reported is a part of a major study on the 'Winter Injury' or 'Die-Back' of the Persian walnut (*Juglans regia*), certain phases of which were presented in 1919 (Batchelor and Reed<sup>1</sup>). The present publication will present data on the seasonal variations in soil moisture and on various factors which affect soil moisture.

We shall endeavor to show the extreme degree to which orchard soils in semi-arid regions may become desiccated at the end of the growing season, and to point out how this dry condition may persist during winters of light rainfall.

The M. Steinburg walnut grove lying one mile south of Hemet, Riverside County, California, was chosen for this study. At the beginning of the experiments, in the fall of 1918, this grove was typical of many which had suffered severely from winter injury. The

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grove is composed of seedling Santa Barbara softshell trees planted 44 feet apart each way. The trees were twelve years old in the spring of 1918. This grove received no irrigation until the fall of 1918. The development of a young walnut grove under dry-farm conditions is rather exceptional in the Hemet region, and the lack of irrigation may possibly be the reason why the grove under discussion exhibited an extreme case of winter injury.

The soil is an alluvial fine sandy loam with a low organic content. In general the subsoil is somewhat finer than the surface soil. This change in the fineness of the soil is gradual and perceptible to a depth of 12 feet. A well drilled in the grove soon after the experiment started showed the nearest ground water to be 84 feet from the surface.

## CLIMATIC FACTORS AFFECTING SOIL MOISTURE

### RAINFALL IN THE HEMET REGION, AND PENETRATION OF RAIN AND IRRIGATION WATER

The walnut grove in which the observations were made is located about four miles from a station of the U. S. Weather Bureau at San Jacinto. Records of rainfall kept since 1886 are available and may be used, although the precipitation at the walnut grove is, on account of neighboring topography, possibly less than at the official Weather Bureau Station. The records of rainfall referred to on the following pages were taken one mile from the grove by the Lake Hemet Water Company during the progress of the experiment. The mean seasonal precipitation for the twenty-seven years from 1892-93 to 1918-19, inclusive, is 13.2 inches, with a range of between 7.9 and 18.9 inches. The monthly distribution of rainfall is shown in table 1.

TABLE 1  
MEAN MONTHLY RAINFALL AT SAN JACINTO (MEAN OF 26 YEARS)

	Inches		Inches
July.....	.13	January.....	2.97
August.....	.21	February.....	2.28
September.....	.17	March.....	2.71
October.....	.66	April.....	1.12
November.....	1.07	May.....	.42
December.....	1.42	June.....	.03

The mean number of days on which there was precipitation of .01 inch or more at San Jacinto is 37. December had an average of four days on which rain fell, January and March had an average of seven, and February of six.

We may summarize the rainfall situation by saying that the grove under consideration is located in a semi-arid district which receives a mean seasonal rainfall of about 13 inches, most of which usually falls in the months of December, January, February, and March. Irrigation is practiced during the growing season, although this particular grove was not irrigated until the autumn of 1918, at which time the trees were twelve years old.

#### EVAPORATING POWER OF THE AIR

##### 1. Sunshine.

The duration of sunshine is related to loss of water in at least two important ways, the concomitant effect of heat and the use of water in photosynthesis. The Hemet region is characterized by almost complete freedom from clouds or fog during the growing season, and the ratio of sunshine received to the amount possible is high.

##### 2. Temperature.

This walnut grove is situated in a district characterized by high summer temperatures and a high evaporation rate. The summer temperatures are somewhat higher than those prevailing in the large walnut districts nearer the Pacific coast. Frequent comparisons of temperatures in the grove with those given by the official observer at San Jacinto showed little difference between the two. We may therefore refer to the official thermometric readings at San Jacinto, where observations have been taken for more than sixteen years. Some of the important temperature data are summarized in table 2, which gives monthly and annual averages together with the highest and lowest temperatures recorded in twenty-three years.

These figures show that the prevailing temperatures are in a way characteristic of conditions in southern California, although somewhat higher than those commonly found in other walnut-growing districts. The determination of the 'mean temperature,' either for the month or for the year, is of little importance in this locality because of the characteristically wide ranges of the daily temperatures. We may, therefore, consider the monthly maxima and minima.



The monthly maximum temperatures are rather high throughout the year, especially during the summer months. In June and the three following months the maximum temperatures are 15 to 20° higher than in the coastal regions of southern California. When the temperature rose to 115° in June, 1917 (the highest temperature ever recorded in this region), there was a small amount of burning on the leaves and young growth of walnut trees. Temperatures of 108° and 110° during the summer were frequently recorded by the instruments

TABLE 2

TEMPERATURE DATA FROM UNITED STATES WEATHER BUREAU RECORDS AT  
SAN JACINTO (IN DEGREES FAHRENHEIT)

Month	Mean tempera- ture	Mean max. tempera- ture	Mean min. tempera- ture	Mean range	Highest tempera- ture	Lowest tempera- ture
January.....	48.8	65.2	35.8	29.4	90	7
February.....	52.2	68.0	37.2	30.8	93	19
March.....	54.9	71.0	40.4	31.6	102	23
April.....	59.5	77.3	44.7	32.6	101	27
May.....	63.8	80.0	48.3	31.7	109	32
June.....	71.4	90.9	53.8	37.1	115	37
July.....	76.8	96.8	59.2	37.6	111	44
August.....	76.4	96.6	58.4	38.2	109	43
September.....	71.6	91.8	53.6	38.2	110	38
October.....	64.2	82.3	47.2	35.1	103	30
November.....	56.8	74.4	39.8	34.6	99	21
December.....	50.3	66.2	34.4	31.8	89	20
Annual.....	62.2	80.0	46.0	34.0	.....	.....
No. of years observed.....	27	16	16	16	23	23

kept in this grove in standard U. S. Weather Bureau shelters. These individual high temperatures have little influence on the monthly averages, especially when several years are averaged together. In individual years the monthly averages may be somewhat higher than those given in table 2; for example, in July, 1920, the mean monthly maximum in this orchard was 103.5° F. There was no damage to the trees as a result of this high temperature. The mean minimum temperatures by months are from 30° to 40° below the maxima for the corresponding time. Indeed, the wide ranges in daily temperatures are very characteristic of these semiarid regions. On occasional days the temperature range may be as great as 50° F. This fall of temperature during the night is an important factor in the activity



of all vegetation, especially of arborescent plants, because it gives an opportunity for equilibrium to be reestablished throughout the tree, after the depletion of water from the transpiring parts during the day. During the hottest part of the day the transpiration may be so rapid from the upper parts of the tree that the water-conducting system is unable to supply enough water to equalize the loss. The lower temperatures during the night, by diminishing the rate of transpiration, are favorable to a more uniform distribution of water throughout the tree.

The data in the last two columns of table 2 show the extremes of temperature which have been registered by the San Jacinto observer over a period of twenty-three years. It is evident that there are days even during the winter when the temperature is high enough to cause rapid evaporation during a few hours each day. Observations reported in another paper<sup>1</sup> showed that these trees do often suffer from desiccation during winters of scanty rainfall. The evaporation through the thin cortical layers of the young twigs is great enough under such conditions to reduce their water content to a serious degree and may result in their death. The injury from this cause is unduly severe if, as often happens, these high temperatures occur simultaneously with periods of low humidity and high wind velocity. The chart on page 12 of the publication cited<sup>1</sup> illustrates a condition which frequently occurs in walnut groves where the amount of water in the soil during the winter is dangerously near the critical point. The walnut trees near the flume which received more irrigation water during the summer suffered least from 'die-back.' The injury was progressively more severe as the distance from the flume increased, because the amount of soil water was progressively less.

### 3. Humidity.

The Hemet district is characterized by low atmospheric humidity during most of the growing season. Under these conditions there is a rapid loss of water from the soil and trees. While the high evaporating power of the air is conspicuous to the most casual observer, and its effects are quickly noticed, it is difficult to make quantitative measurements of the condition which is commonly expressed by the term 'aridity.'

We know of no determination of the evaporation of water from a free surface in the Hemet district, but we may gain a fairly trust-

worthy idea of its rate from the data published by Russell.<sup>5</sup> His tables show evaporation of 43.3 inches at Sacramento and 56 inches at Fresno during the eight months from April 1 to November 30. If we assume that the evaporation at Hemet is 48 inches for this period, we shall probably not be far wrong. During this period the average rainfall for the Hemet-San Jacinto region is 3.81 inches. Using these

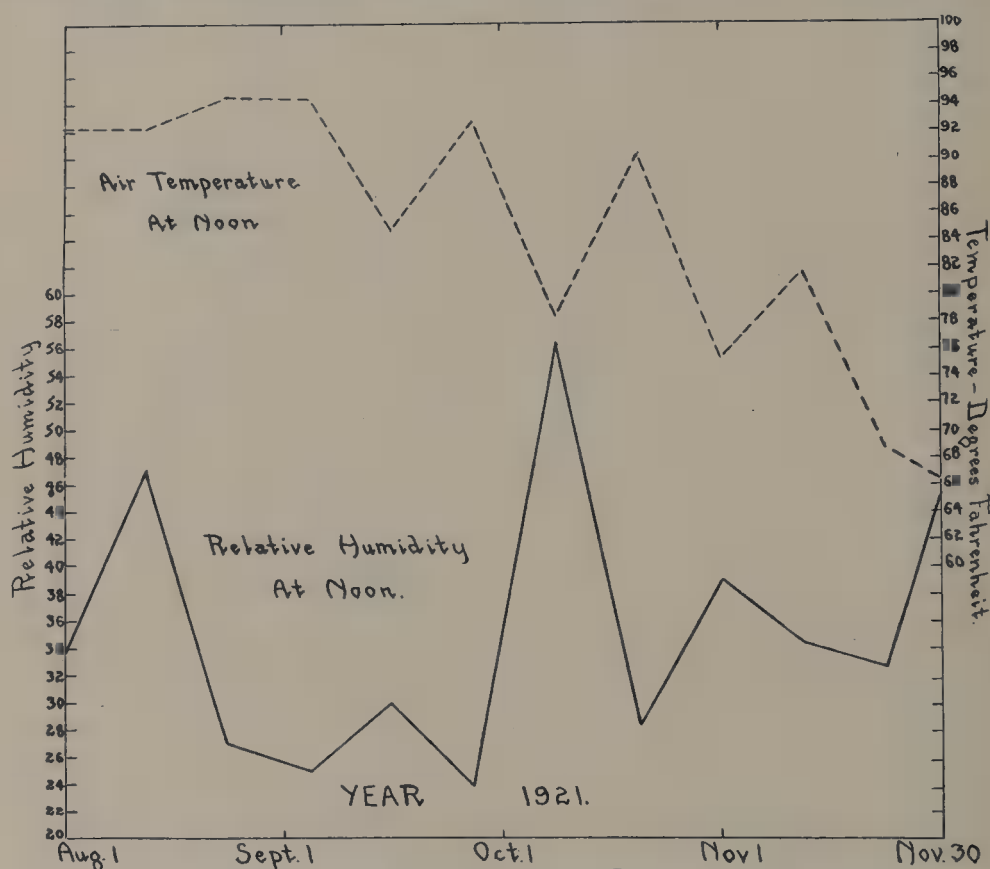


Diagram 1. Relative humidity and temperature at Hemet, August 1 to December 1, 1921.

figures, we see that the ratio of rainfall to evaporation is .08. This period, including two-thirds of the calendar year, is certainly marked by high evaporation and shows one aspect of the climatic conditions which govern plant life in this region.

Determinations of the vapor pressure of the air at Hemet were made during the latter part of the season of 1921 by means of wet and dry bulb thermometers. The determinations are expressed as mean relative humidity for ten-day intervals and are shown in diagram 1. They show that, for most of the season subsequent to

July, the moisture content of the air was very low. The one outstanding exception occurred early in October. It followed a precipitation of 2.95 inches of rain from September 30 to October 2, and was simultaneous with a depression of the mean midday temperature. Another rain on October 23 and 24, which gave a precipitation of .43 inch, also caused an increase in the relative humidity and a fall in mean midday temperature.

These figures give a picture of the evaporating power of the air at a time of the day when evaporation is probably close to the maximum. It is certain that the rate of evaporation during most of the day is much below this figure. During the night the trees have an opportunity to recover from water deficit incurred during the middle of the day.

#### 4. Winds.

Winds in the Hemet region have a well-marked influence upon evaporation. During the summer and fall months there is usually a moderate westerly wind every day. This is the 'coast breeze' which blows from the Pacific Ocean to the hot interior deserts. Although the wind comes from the ocean, its relative humidity is not high, at least by the time it reaches the Hemet district, which is 45 miles from the coast. This daily coastal wind undoubtedly increases the evaporation from both soil and trees.

Evaporation is also increased by irregular east or north winds which come for periods of several days during the fall or early winter months. These winds, coming from the inland deserts, are powerful desiccating agencies. The relative humidity often ranges from 13 to 20 per cent for twenty-four to forty-eight hours at a time, during the prevalence of these northerly winds. Although the trees are partially or wholly bare of leaves at this season, there is nevertheless an appreciable loss of water through the thin bark of the young growth.

#### LENGTH OF GROWING SEASON

The time between the last killing frost in the spring and the first killing frost in the fall, generally designated as the 'frostless season,' corresponds fairly closely with the growing season for walnuts.

The Weather Bureau records show that the average length of the frostless season at San Jacinto for eighteen years is 254 days, with a

minimum of 199 and a maximum of 301 days. For the three years during which our observations were being made in this orchard at Hemet the dates between killing frosts were:

1919, March 21 to November 28	(252 days)
1920, March 27 to December 4	(252 days)
1921, April 6 to November 18	(227 days)

The length of the first two seasons was very nearly the mean, but the last season was considerably shorter.

## CONDITION OF TREES AND SOIL AT THE BEGINNING OF THE EXPERIMENTS

The majority of the trees in the several plots (diagram 2) were severely killed back by winter injury during the winter of 1917-18. That the cause of the winter injury in this grove during this season was due to winter drought seems reasonably certain. The first winter rain occurred January 13, 1918, when .43 of an inch fell. The second rain of .25 of an inch occurred on January 25, 1918.

The last rain of 0.3 of an inch or more preceding that of January 13, 1918, fell on April 17, 1917 (with the exception of a thunderstorm on July 27 which gave 1.87 inches). Thus 270 days had elapsed between rains that were of sufficient amount to affect the moisture content of the soil in any great portion of the root zone. The shower in July probably reached a few of the surface roots in the first foot of soil, which was in an air-dried condition previous to the rain. Not until January 26, 1918, did enough rain fall to moisten the surface foot of soil. On this date 1.14 inches of rain fell.

The extreme dryness of the soil in this grove before the winter rains began can be realized when it is recalled that there were twenty-two walnut trees per acre, twelve years old, growing on the land under dry-farm conditions where the normal rainfall of 13 inches comes almost exclusively during the dormant period of the walnut tree. Thus in the absence of rain from July to January 13, the soil-moisture content of the root zone was reduced to a point seldom reached under cultural conditions.

Soil samples were taken October 30, 1917, when the leaves on the trees were turning yellow and a portion of them had fallen. At this

time the surface foot of soil was dust-dry and showed a moisture content of only 0.7 per cent. The condition of the subsoil is shown by the ratio given in table 3.

As mentioned above, such dryness of the subsoil as herein reported is seldom observed under cultural conditions. In fact the results of the preliminary observations were so extreme that it seemed as though an unnoticed error must have occurred in weighing or calculating. Repetitions, however, confirmed these findings.

TABLE 3

THE SOIL MOISTURE AND ITS RELATION TO THE HYGROSCOPIC COEFFICIENT AT THE  
END OF THE 1917 GROWING SEASON

Depth	Hygroscopic point	Moisture observed	Ratio
	Per cent	Per cent	
2nd foot.....	2.44	1.31	0.54
3rd foot.....	2.26	1.74	0.77
4th foot.....	2.74	2.34	0.85
5th foot.....	4.30	2.97	0.69

Most of the soil-moisture studies made by previous workers under field conditions have dealt with that portion of the soil moisture which is above the wilting point, because they have not been made under the extremely arid conditions which may be observed in regions like that here described. Alway,<sup>2</sup> however, has reported studies in which the soil moisture was below the hygroscopic point. He gives data upon the soil of an abandoned olive orchard in Arizona, of the prairies of southwestern Nebraska,<sup>3</sup> and of cylinders in which desert legumes had been grown under controlled conditions. Regarding the condition of these perennial desert legumes when the observations were made the author reports, "In none were all the plants dead when the cylinders were opened but in the case of each all the tips had died and nearly all the leaves had fallen, the most vigorous plant in each retaining only from five to seven compound leaves." "In experiments with perennial desert legumes the plants remained alive after the water content had fallen slightly, but distinctly, below the hygroscopic coefficient . . . " Apparently the moisture conditions in the dry-farm walnut grove are similar to those in Alway's cylinders in which desert legumes were grown. As that author has suggested in the case of the desert plants, although there is no evidence of any ability on the



part of the walnut to use the last portion of free water *for growth*, there is an indication that the moisture between the wilting point and the hygroscopic point, and even some of the water below the



Fig. 1. A general view in the Steinburg walnut grove in May, 1918, showing the injury which resulted from a lack of soil moisture during the preceding winter.

hygroscopic point, may have a very high value for the maintenance of the life of the perennial and tree crops.

As heretofore noted, in the spring following the dry winter of 1917 many of the trees were dead in the uppermost branches and some of them killed back nearly to the main scaffold limbs. The distribution of the injured trees in the grove is given in diagram 2, while the nature of the injury on some of the individual trees is shown in figures 1 and 2.



Fig. 2. A badly injured tree in the Steinburg grove, showing how the young branches tend to die back as a result of winter drought.



The grove was dry-farmed during the summer of 1918 which followed a total rainfall of 13.46 inches for the entire rainy season preceding. In the following November the owner installed an irrigation pipe line and the grove was given a light irrigation of 3.6 acre

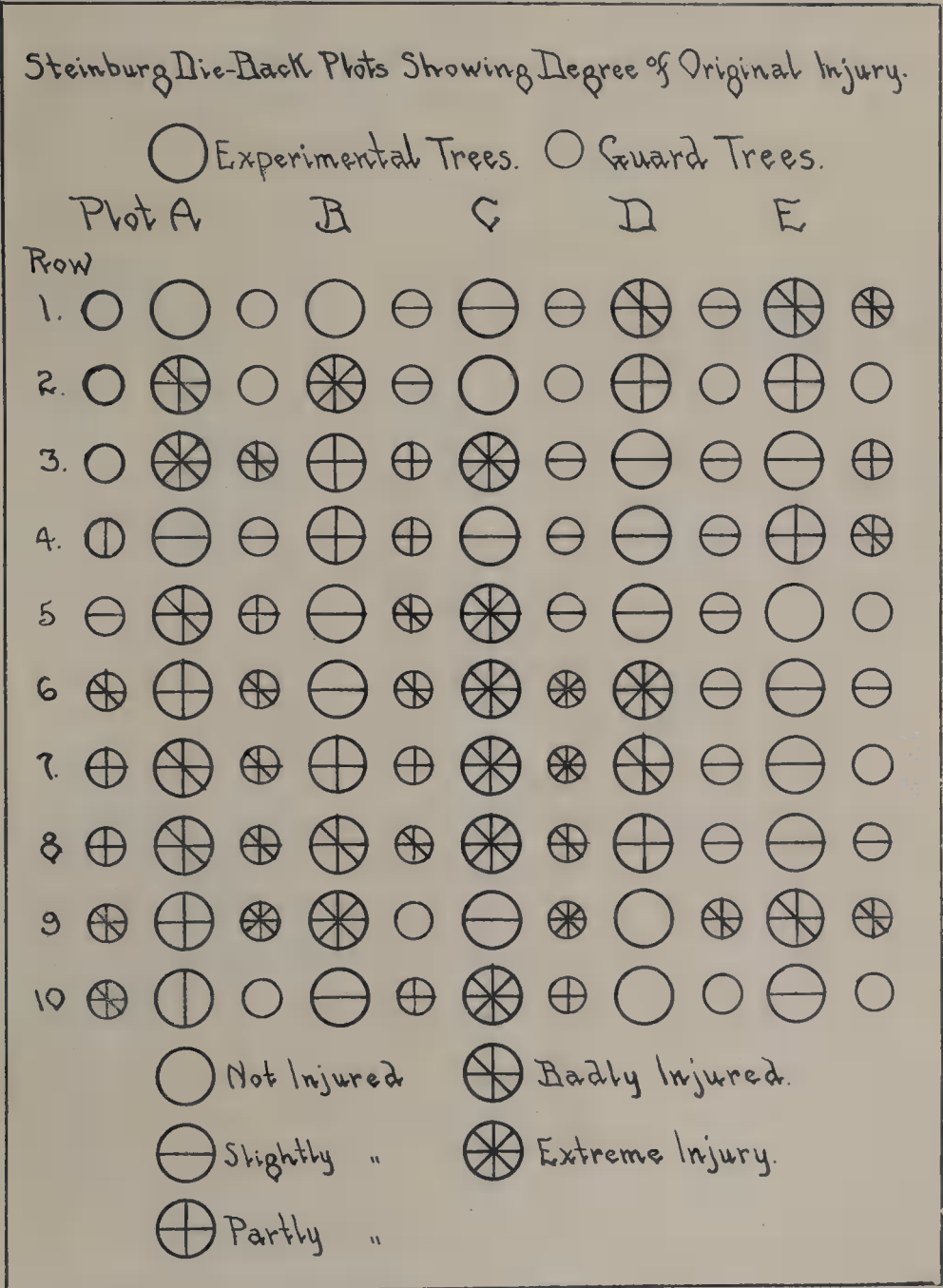


Diagram 2. Distribution of injured trees in Steinburg grove, 1918.

inches per acre, after which it passed under the control of the Citrus Experiment Station and was irrigated as will be described later.

Since the winter of 1917-18 the rainfall has commenced early and has been sufficient in amount to prevent drought injury; thus the seasons have not been favorable to a study of extreme winter drought conditions as related to the growth of winter-irrigated walnuts. This may therefore be a somewhat incomplete report of progress. Inasmuch, however, as the work is to be discontinued on the Steinsburg grove and taken up on the Citrus Experiment Station grounds, it seems best to report progress to date.

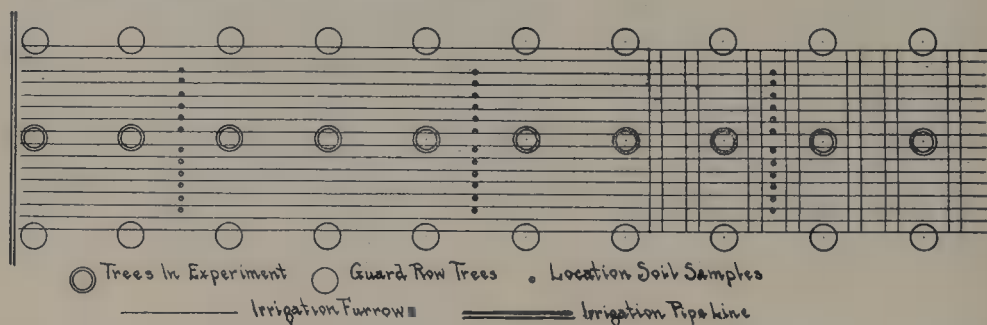


Diagram 3. Map of a typical plot showing location of irrigation furrows, locations from which samples were taken, and relative positions of experimental and guard trees.

## METHODS OF PROCEDURE

### PLAN OF PLOTS

Each trial plot was laid out to include ten trees in a single row with guard rows between plots. The entire area from guard row to guard row was irrigated, sampled, and in every way regarded as belonging to the adjacent experiment row. See diagram 3.

### IRRIGATION

The irrigation water was measured over a rectangular weir and applied in deep furrows 440 feet long; 8 furrows were used in each interspace of 44 feet, with the lower portion of the plot cross-furrowed. The method of cross-furrowing retarded the velocity of the water and increased its depth in the furrows, thus largely equalizing the amounts of water received by the upper and lower ends of the plots.

Water was applied at the rate of 15 southern California miner's inches (0.3 of a second foot) per acre. This is a larger stream of water per acre than is commonly used in the Hemet-San Jacinto section. Because of the sandy nature of the experimental plots, however, the use of the stream mentioned was in harmony with good irrigation practice.

TABLE 4  
IRRIGATION WATER APPLIED TO THE PLOTS (IN ACRE INCHES PER ACRE)

Dates	Plots				
	A	B	C	D	E
<i>1918</i>					
November 8-9.....	3.6	3.6	3.6	3.6	3.6
December 18.....	5.7		4.0		9.7
<i>1919</i>					
April 16.....		1.8			
May 14.....		1.8			
June 19.....	4.2	1.8	4.2	4.2	4.2
July 16.....	4.2	1.8		4.2	4.2
August 13.....	4.2	1.8	4.2	4.2	4.2
September 17.....		1.8			
October 15.....		1.8			
December 11-13.....	6.6		4.2		9.7
	28.5	16.2	20.2	16.2	35.6
<i>1920</i>					
March 16-17.....	7.2				
April 20.....		1.8			
May 18.....		1.8			
June 15.....	4.2	1.8	4.2	4.2	4.2
July 20.....	4.2	1.8		4.2	4.2
August 10.....	3.2	1.8	4.2	4.2	4.2
September 15.....		1.8			
October 13.....		1.8			
November 30.....	13.1		4.2		9.7
	31.9	12.6	12.6	12.6	22.3
<i>1921</i>					
April 18.....		1.8			
May 16.....		1.8			
June 14.....	4.2	1.8	4.2	4.2	4.2
July 19.....	4.2	1.8	4.2	4.2	4.2
August 9.....	4.2	1.8		4.2	4.2
September 13.....		1.8			
October 11.....		1.8			
December 13-15.....	6.3		4.2		6.5
	18.9	12.6	12.6	12.6	19.1

In general the water-holding capacity of this soil gradually increases with the depth. The hygroscopic point of the surface soil varies from 2 to 3 per cent, whereas that of the sixth and seventh foot varies from 3 to 6 per cent. There is one abrupt change in soil type in plot D. The fourth foot in one of the transverse sampling areas consists largely of coarse sand the hygroscopic point of which is only 1.98 per cent.

Since the experiment was installed for the purpose of acquiring information on the relation between soil water and die-back, the amounts of water applied to the several plots and the time of their application were varied. The schedule of applications is given in table 4. It will be seen that this plan of irrigation afforded an admirable opportunity to observe the water content of the soil as affected by the growth of the trees and by the application of different quantities of water. Plots B, C, and D in the last two years received the same total quantity of irrigation water (12.6 inches), the customary amount in the Hemet district, but the time of application was varied in the different cases. Plots A and E were given considerably more water in the winter than the other plots.

So far as the trees are concerned, however, the amount of water in the soil is of more importance than a record of the amount of water applied. Determinations of the amount of water in the soils of the various plots were made before and after each irrigation and at such other times as were judged necessary.

#### SOIL SAMPLING

As noted in diagram 3 the samples were taken across the upper, middle, and lower end of each plot. At each transverse area samples were taken for each foot separately, down to and including the seventh foot, and each sample consisted of a composite made up of six cores from the soil tube.

A moisture determination was made on each of the composite samples and the three results were averaged to obtain the mean moisture content for each footlevel of the plot.

## METHOD OF EXPRESSING RESULTS

## 1. Soil Moisture Data.

In the following discussion the soil moisture content is stated in the form of a ratio to the hygroscopic coefficient, following the usage of Alway and co-workers,<sup>3</sup> whose comprehensive tables and discussions might well serve as an example to other workers. With a tabulation of the hygroscopic coefficients in tables 7 and 8 preceding the respective ratios, the reader may visualize the type of soil worked with as well as its relative moistness.

We have usually compared our data on the moisture content of the soils with the hygroscopic coefficient rather than the wilting-point coefficient. There seemed to be two good reasons for such a procedure: first, the trees often did not wilt when the soil moisture in the root zone was below the 'wilting point,' and second, the comparisons of moisture content with the wilting point would frequently involve the use of minus quantities.

## 2. Reliability of the Hygroscopic Coefficient.

The labor involved in sampling the several plots twice per month throughout most of the year made the determination of the moisture equivalent and hence of the hygroscopic coefficient for each respective composite of six cores a practical impossibility. The hygroscopic coefficients here presented are the mean values for a series of six determinations made in the respective areas, each determination representing a composite sample taken entirely independently of every other at an interval of at least thirty days. The determinations for the six respective samples were made by four different people. The personal and time elements, as well as the error in sampling, were therefore somewhat reduced in obtaining this mean as compared with the determination of six samples from the same composite, or of samples from different composites taken by the same person simultaneously. The six determinations may thus be considered as six attempts to measure the same thing, viz., the hygroscopic point of a certain foot section of a given plot.

A statistical inquiry may be profitably applied to these data to test their reliability as compared with the mean of an infinite number of determinations. The tables of 'Student'<sup>6</sup> serve as a ready means of applying statistical methods to this inquiry. As this author so aptly states: "Any experiment may be regarded as forming an indi-



vidual of a population of experiments which might be performed under the same conditions. A series of experiments is a sample drawn from this population." With this viewpoint in mind it becomes of interest to determine the reliability of a mean of a series of six determinations of the hygroscopic coefficient. The third foot in plot A in the sampling area nearest the pipe line will serve as a fair example. Following 'Student's' procedure the example works out as follows:

RELIABILITY OF THE MEAN OF THE HYGROSCOPIC COEFFICIENT

V	D	D <sup>2</sup>
3.91	.33	.1089
3.80	.22	.0484
3.06	— .52	.2704
3.24	— .34	.1156
3.89	.31	.0961
3.57	— .01	.0001
M = 3.58		6) .6395
		.1066

$$S. D. = \sqrt{.1066} = .3265$$

The probability that the true mean does not differ from the mean of the sample by more than  $\pm .3$  per cent moisture may be found as follows:

$$Z = \frac{.3}{.3265} = .9$$

In Student's tables when  $Z = .9$  and  $N = 6$ ,  $P$  is .9498. Subtracting,  $1.0000 - .9498 = .0502$ , which may be taken to mean that the chances are .9498:.0502 that the true mean does not exceed the mean of the sample by more than .3 per cent moisture.

In our problem, however, we are concerned with a probability that the true mean is just as liable to lie below as above the mean of the sample. Thus we proceed farther to obtain these odds as follows:  $.0502 \times 2 = .1004$ . Subtracting as before,  $1.0000 - .1004 = .8996$ . The odds that the true mean does not vary from the mean of the sample by more than  $\pm .3$  per cent are .8996:.1004, or practically 9 to 1.

Although odds of 9 to 1 are not considered a 'reasonable certainty' in statistical studies, such odds do indicate that the use of the mean of the hygroscopic coefficients is probably as reliable within the limits of  $\pm .3$  per cent moisture as most comparisons, subject to the error of soil sampling.



RESULTS AND DISCUSSIONS

PENETRATION OF RAIN AND IRRIGATION WATER

The soil on which this grove is located absorbs water readily, and there is practically no run-off, even during seasons of heavy precipitation. The rate of water penetration is roughly indicated by table 5,

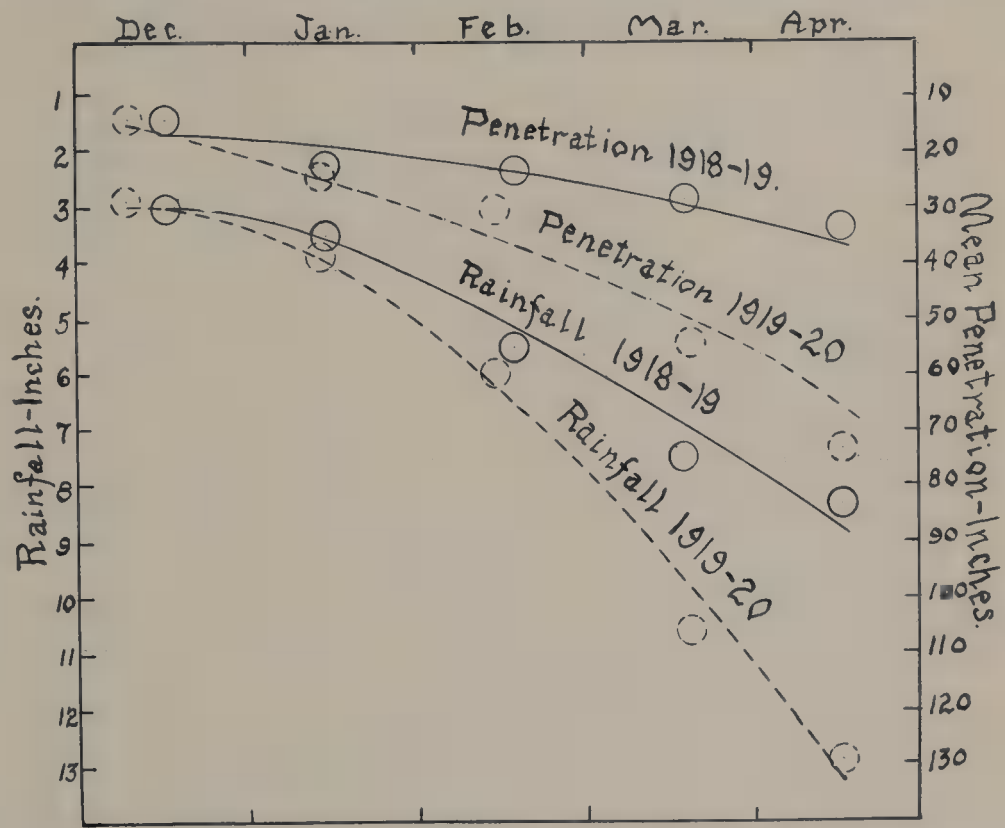


Diagram 4. Relation of rainfall to its penetration in the grove in two seasons.

showing the observations made during the winters of 1918-19 to 1920-21, inclusive. Diagram 4 represents the relation of rainfall to penetration as based on two-years' observation.

The moisture penetrates this soil from 4 to 6 inches for each inch of rain. The factors responsible for this relatively small penetration seem to be: first, the extreme initial dryness of the soil; second, the small amount of rain falling in many individual storms; third, a rate of evaporation between storms so rapid that much of the water passes into the air before another storm occurs.

The penetration of irrigation water may be illustrated by plot A as observed during the winter of 1919-20. The rain to December 9 was 2.87 inches and it had penetrated 10 inches on plot A. The plot was then irrigated with 6.56 acre inches per acre, and 8 days later the moisture penetration in 18 holes averaged 54 inches. The soil moisture had been lowered, therefore, 6.7 inches per acre inch of water applied. This is somewhat less penetration than Harding<sup>4</sup>

TABLE 5  
RAINFALL AND DEPTH OF PENETRATION IN PLOTS B AND D (IN INCHES)  
1918-1919

Date	Dec. 16	Jan. 15	Feb. 17	Mar. 18	April 16	Average penetration per inch of rainfall
Rainfall to date.....	2.99	3.45	5.46	7.47	8.25	
Penetration in dry plots..	14	22	23	28	33	4.0
		1919-1920				
Date	Dec. 9	Jan. 13	Feb. 18	Mar. 18	April 19	
Rainfall to date.....	2.87	3.91	5.96	10.58	12.88	
Penetration in dry plots ..	10	24	30	58	73	5.7
		1920-1921				
Date	Dec. 15		Feb. 2	Mar. 8	April 18	
Rainfall to date.....	1.63	.....	4.60	5.12	6.20	
Penetration in dry plots ..	18	.....	24	25	37	5.9

Mean 5.2

reports for a similar amount of water. Harding, however, was dealing with movement of soil moisture under ordinary irrigation practice where the content seldom drops below the wilting point, whereas we are considering the winter irrigation of a soil in which the initial soil moisture was close to the hygroscopic point 120 days after the last irrigation.

The summer irrigation which was given at a time when the moisture was close to the wilting point penetrated deeper and harmonizes

more closely with Harding's observations. This is best illustrated by plots A and C following August irrigation of 1920. Table 6 shows the amount of water applied, the relative dryness of the soil before irrigation and the average penetration per acre inch of water. In plot A, with the moisture of the first 60 inches of soil considerably above the wilting point, the average penetration of 3.18 acre inches of water was 55.9 inches or 17.6 inches per acre inch of irrigation water. Pot C, which was much dryer and in fact had a moisture content nearer the hygroscopic point than the wilting point, after an irrigation of 4.2 acre inches, showed an average penetration of 43.3 inches or 10.3 inches per acre inch of irrigation water.

TABLE 6

DEPTH OF PENETRATION OF IRRIGATION WATER ON PLOTS A AND C, AUGUST, 1920

Plot	Water applied	Soil moisture before irrigation (upper 60 inches)	Average penetration per acre inch
	Acre inches* per acre	Ratio to hygroscopic point	Inches
A.....	3.18	1.66	17.6
C.....	4.20	1.17	10.3

\* Amount of water accounted for by rise in moisture content of soil 10 days after irrigation.  
A 2.128 acre inches per acre.  
C 2.641 acre inches per acre.

It may be instructive to examine the records of plot A and to note how the applications of water affected the moisture content of the soil to the depth of 7 feet. Diagram 5 represents graphically the per cent of water in the soil in one-foot sections from December, 1919, to the end of October, 1920, expressing the water as a per cent of the weight of dry soil. Between August 1 and December 1, the precipitation amounted to 1.84 inches at Hemet, but it had fallen in scattering showers and had not appreciably affected the water content of the soil below the first foot. At the beginning of the observations the soil moisture below the first foot was very close to the hygroscopic coefficient. After the irrigation of 6.6 inches on December 11-13 there was a perceptible increase in the water content of the second, third, fourth, and fifth foot layers of soil, as determined from samples taken six days after irrigation. At the time the soil was next sampled, in March, the water content of the deeper layers had

increased to about the same per cent as that of the upper layers. This was due both to the steady downward movement of water and to the addition of 6.56 inches of rain after January 1. The heavy irrigation (7.2 inches) given on March 16 and 17 appreciably raised the water content of the deeper layers of the soil and to a less extent of the

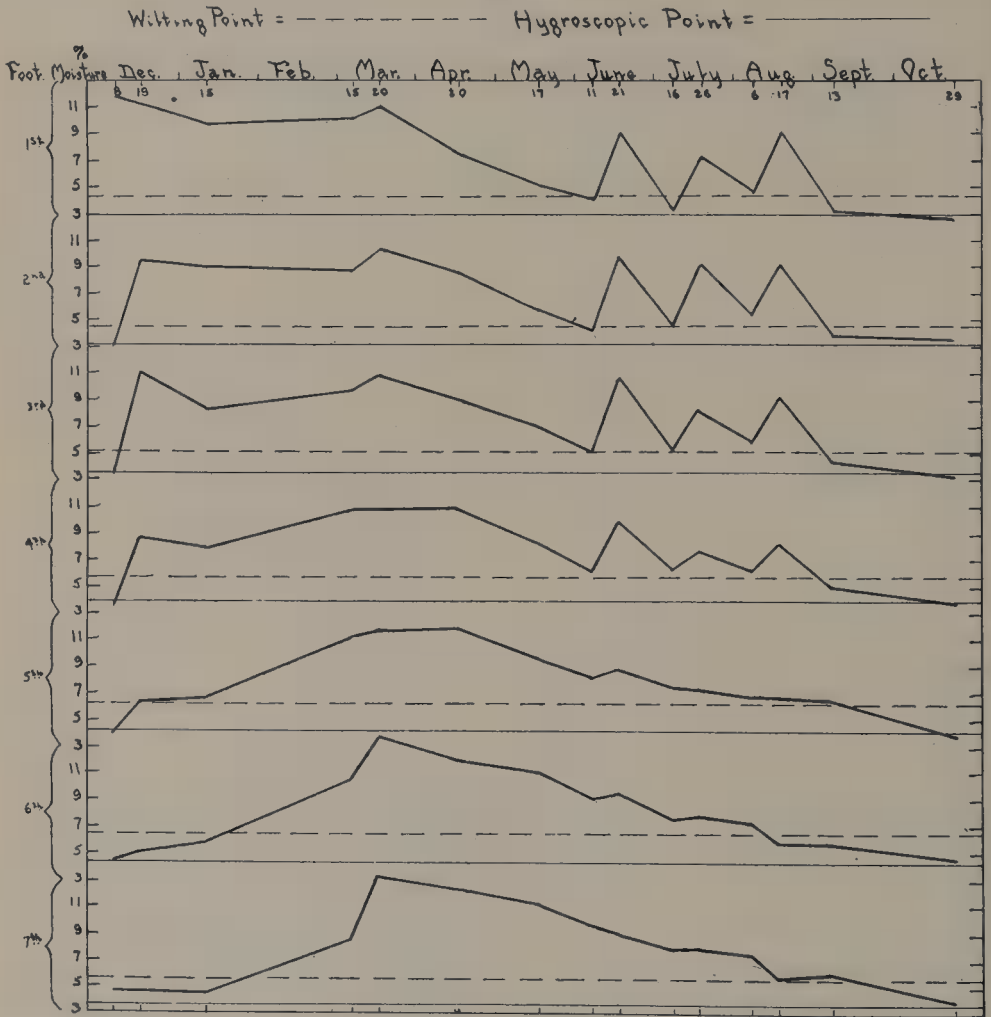


Diagram 5. Seasonal variations in moisture content of the upper seven feet of soil in plot A from December, 1919, to October, 1920. The horizontal line represents the mean hygroscopic coefficient of the samples at each level, and the broken line represents their wilting point coefficient.

surface layers. After a precipitation of .93 inches on March 23 there were no more rains of consequence and the soil water content steadily diminished until the next irrigation, owing to the removal of water by the trees during the period of rapid growth characteristic of spring and early summer. When sampled on June 10, the soil water of the

first four foot-layers was practically at the wilting point. In other words, the trees had removed practically all of the water 'for growth' from the zones in which most of the active roots are located. The application of 4.2 inches of irrigation water in June and July, and of 3.2 inches in August temporarily raised the water content of the first four foot-layers of soil, but made little difference in the water content of the lower layers.

The depth of penetration of like amounts of irrigation water varied from year to year according to the rainfall. This can be readily understood by noting the rainfall penetration shown in diagram 4.

#### EFFECT OF IRRIGATION ON SOIL MOISTURE

The relation of soil moisture during the winter months to the need of irrigation the following summer or the following growing season is of both practical and scientific interest. Table 7 gives the moisture present in plots A and D from March, 1920, at monthly intervals until October. Both plots received practically the same irrigation during June, July, and August. Plot A received winter irrigation of 13.8 acre inches per acre, while plot D was not winter-irrigated. The winter rains amounted to 13.74 inches and penetrated 73 inches on plot D. Although plot A showed a considerably greater amount of soil moisture at the beginning of the growing season, there was no significant difference in the two plots by the middle of July. This table and table 8 show that there is no 'hold-over' of soil moisture from one season to another in the zone of greatest root development. It may also be concluded from this table that the effect of heavy winter irrigation will not persist beyond the middle of the growing season under the condition in this orchard. From this time until the beginning of the dormant period, the irrigation needs are the same as though no winter water had been applied. This may not apply to the subsoil where relatively few roots are developed, a depth from 8 to 20 feet from the surface, provided any of the irrigation water reaches such depths; the rainfall seldom reaches a depth of more than 4 or 5 feet in the district in question. Slight irregularities had gradually developed in plots A and D when the data in table 7 were taken. Since the former plot had been heavily winter-irrigated for the two years previous, the trees had grown somewhat larger and no doubt made somewhat greater demands on the soil moisture than those in plot D.

TABLE 7

COMPARISON OF SOIL MOISTURE IN PLOT A RECEIVING HEAVY WINTER IRRIGATION OF 13.8 ACRE INCHES PER ACRE  
AND PLOT D WHICH WAS NOT WINTER-IRRIGATED IN 1919-1920

Soil moisture expressed as a ratio of the hygroscopic point

Ft.	Hygro. point		March			June			July			August			September			October		
	A	D	A	D	Dif.	A	D	Dif.	A	D	Dif.	A	D	Dif.	A	D	Dif.	A	D	Dif.
1.....	3.05	2.45	3.67	3.30	0.37	1.39	1.08	0.31	1.11	0.94	0.17	1.61	1.45	0.16	1.12	0.96	0.16	0.92	1.11	-0.19
2.....	3.17	2.43	3.41	3.08	0.33	1.40	1.34	0.06	1.47	1.66	-0.19	1.74	1.87	-0.13	1.24	1.37	-0.13	1.12	0.95	0.17
3.....	3.61	2.83	3.05	3.17	-0.12	1.43	1.71	-0.28	1.50	1.72	-0.22	1.67	1.91	-0.24	1.26	1.51	-0.25	0.94	1.08	-0.14
4.....	3.97	2.77	2.78	3.17	-0.39	1.57	1.55	0.02	1.61	1.88	-0.27	1.64	2.08	-0.44	1.28	1.52	-0.24	0.95	1.09	-0.14
5.....	4.21	4.56	2.80	2.40	0.40	1.95	1.63	0.32	1.78	1.63	0.15	1.64	1.78	-0.14	1.34	1.46	-0.12	0.92	1.02	-0.10
6.....	4.42	5.71	3.12	1.80	1.32	2.08	1.69	0.39	1.76	1.88	-0.12	1.69	1.83	-0.14	1.30	1.58	-0.28	1.06	1.07	-0.01
7.....	3.79	5.32	3.53	1.61	1.92	2.56	1.78	0.78	2.13	2.13	0.00	2.02	1.93	0.09	1.64	1.70	-0.06	1.03	1.33	-0.30
Mean	3.75	3.72	3.19	2.62	0.55	1.77	1.54	0.23	1.62	1.69	-0.07	1.72	1.84	-0.12	1.31	1.44	-0.13	0.99	1.09	-0.10



TABLE 8  
SOIL MOISTURE IN THREE PLOTS EXPRESSED AS A RATIO OF THE HYGROSCOPIC POINT  
(The hygroscopic points stated below are the means of six different sets of samples and determinations for the respective foot-samples.)

Depth of foot-section	Plot A				Plot B				Plot C			
	Soil moisture				Soil moisture				Soil moisture			
	Hygro. Point	Oct. 1919	Oct. 1920	Sept. 1921	Hygro. Point	Oct. 1919	Oct. 1920	Sept. 1921	Hygro. Point	Oct. 1919	Oct. 1920	Sept. 1921
Upper portion of plot	1	2.79	0.76	1.18	2.43	1.20	2.00	1.50	2.11	1.42	1.13	0.99
	2	2.98	1.03	0.92	2.77	0.88	1.78	1.09	2.39	1.04	1.03	0.94
	3	3.58	0.99	0.92	2.71	1.04	1.59	1.62	2.33	1.14	1.25	1.11
	4	4.09	1.06	0.90	3.39	.....	1.33	1.23	2.69	0.90	1.38	1.36
	5	4.08	1.08	0.97	3.67	0.95	1.05	1.00	4.88	0.88	1.14	0.95
	6	3.85	1.02	0.98	3.38	1.15	1.32	1.17	6.42	0.98	1.06	1.06
	7	3.69	1.08	1.00	3.09	1.09	1.05	1.32	4.87	0.93	1.45	1.19
Middle portion of plot	1	2.85	0.46	0.95	2.52	0.61	1.50	1.11	2.52	1.23	0.76	0.46
	2	2.86	0.88	1.13	2.56	1.09	1.27	1.38	2.44	0.94	0.98	0.96
	3	3.25	1.06	1.12	3.05	1.06	1.10	1.22	2.26	0.99	1.10	0.91
	4	3.50	1.06	0.99	4.08	0.98	1.07	1.22	2.74	0.85	1.07	1.22
	5	3.97	1.06	1.08	4.24	0.92	1.00	1.07	4.30	0.92	1.04	0.97
	6	4.30	0.95	1.08	3.72	1.01	1.04	1.15	4.41	0.92	1.15	1.11
	7	3.46	1.10	1.25	3.88	0.80	1.03	1.13	3.35	1.12	1.79	1.11
Lower portion of plot	1	3.52	0.81	0.61	2.92	1.22	1.23	0.70	2.51	1.19	0.98	0.72
	2	3.67	0.88	0.88	3.04	0.89	1.03	1.26	2.60	0.94	0.90	0.83
	3	3.99	0.98	0.89	3.39	1.23	1.06	1.14	3.01	0.91	1.01	0.99
	4	4.31	0.97	0.91	4.28	1.14	1.09	0.81	4.48	0.95	1.03	0.85
	5	4.58	1.02	1.02	4.59	1.10	1.03	0.93	5.52	0.89	0.99	0.82
	6	5.10	1.12	0.98	4.88	1.07	0.96	0.99	3.68	1.03	1.23	0.98
	7	4.23	1.25	0.94	4.48	0.86	0.91	0.83	2.95	1.05	1.33	1.03
Mean		0.98	1.07	0.99		1.01	1.21	1.14		1.01	1.13	0.98

The percentage of water in the soil of plot A and its variations during the growing season are given in diagram 5. The water content of each of the upper seven feet of soil is stated in relation to the wilting and the hygroscopic coefficients. The diagrams show how the water content of soil lying at various depths is influenced by additions of water. The upper layers of soil respond more quickly to a fall of rain or to an application of irrigation water, but also lose their water more quickly. The loss of water during the summer months is principally due to the absorbing action of the tree roots, which are more densely distributed in the upper four feet of soil in this grove. It will be noted that the water content of each foot-layer except the first was below the wilting point on December 5, 1919; indeed, with the exception of the first and seventh, all were below the hygroscopic point. As the result of an irrigation of 6.6 acre inches per acre, the moisture content of the second, third, and fourth foot-layers rose sharply, but the fifth, sixth, and seventh foot-layers were not raised above the wilting point.

As a result of the winter rains and of another irrigation of 7.2 acre inches the water content of the various layers was much higher when sampled March 31, 1920. The increase was especially marked in the case of the first and the seventh foot-layers. Subsequent samples taken April 19, May 17, and June 11 showed a steady decline in water content. The trees put out new foliage and began active growth the last week in March.

The samples taken on June 11 showed that the water of the layers of soil occupied by most of the tree roots had fallen approximately to the wilting point. Irrigations of 4.2 acre inches per acre in June, July, and of 3.2 in August, materially raised the water content for a brief time, but within thirty days it dropped back to the approximate value of the wilting point. During the entire summer the water content of the fifth, sixth, and seventh foot-layers declined almost continuously, showing but little response to the irrigation water applied. No water was applied to this plot in September or October. When the plot was sampled on October 29, the soil at all levels in the upper seven feet was approximately at the hygroscopic point.

These observations show that in this grove the stored soil moisture from heavy winter rains or irrigation is of importance in the upper root zone only up to the middle of the growing season. The effect of

such moisture may persist in the subsoil where fewer roots are developed, until well past the middle of the growing season. They also show the failure of ordinary medium applications of irrigation water (4.6 acre inches per acre) to maintain the soil moisture during the late summer months.

#### DESICCATION OF THE SOIL BY TREE ROOTS

The dryness to which the soil is reduced\* at the end of each growing season is of vital interest in connection with the possible winter-injury of the trees due to desiccation during seasons of light rainfall.

The soil moisture present at the commencement of the dormant period of the trees is generally very small. Under the conditions which usually prevail, the last irrigation is applied late in August† just before the nuts ripen. The fall and early winter rains have come too early during the years 1919–1921, inclusive, to permit the study of the effect of a winter drought such as occurred in the two years before the irrigation experiment began. The studies here reported are therefore not conclusive proof of the effect of winter drought on walnut trees, but rather indicate the extreme dryness to which the soil is reduced at the commencement of the annual dormant period of the trees. When sufficient rain falls early in the dormant period the trees show no injury from autumn drought. Table 8 shows the moisture content of plots A, B, and C during the falls of 1919, 1920, and 1921.

It is apparent from this table and the discussion in a subsequent paragraph that the wilting point of the soil has no significance for the use of soil moisture by the walnut tree. The hygroscopic point is the critical point at which growth stops and the point at which the tree matures in a perfectly healthy and normal condition. Although this conclusion may not harmonize with many of the soil-moisture studies where annual crops have been used as indicators, it does agree with Alway's studies heretofore mentioned.

The continued dryness of the soil (at the hygroscopic point or below) throughout most of the root zone, is thus a matter of course

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\* The soil moisture content in the following discussion is stated as a ratio to the hygroscopic coefficient.

† The statement above applies to the irrigation practice in the majority of the walnut-growing sections. In the Hemet region most of the walnut groves are irrigated according to the schedule shown for plot B, on page 13.

during winter seasons of scanty rainfall. The same effect is produced in walnut groves in which is growing a winter barley crop which uses the moisture soon after it falls and thus prevents it from reaching the subsoil. Many observations made in barley fields during the winter of 1918-19 showed that 18 to 24 inches was the maximum depth which the rainfall reached, because, with the light occasional rains and the demands of the barley plants, there was no moisture left for deep penetration. During seasons of normal rainfall walnut trees are frequently severely injured by winter drought if intercropped with barley.

During the progress of this experiment a few cases of winter injury due apparently to drought have come to the attention of the authors. These have all occurred in intercropped orchards. Some have occurred in central California and it has been impossible to obtain any knowledge of the soil-moisture conditions which prevailed before the winter rains and the occurrence of the injury. These survey studies have still further strengthened the conclusions reported by the authors in 1919.<sup>1</sup>

Table 9 shows that in two young orchards, one of apricot and one of peach, on Placentia sandy loam, the latter underlain by impervious hardpan, the moisture content had been reduced below the hygroscopic point. The soil samples were taken December 1, 1921, 113 days after the last irrigation. A light shower occurred in October but it affected only the surface of the soil. The trees in these orchards were apparently entering a normal dormant period on December 1. The following spring (1922) these apricot and peach trees bloomed and started their normal spring growth, which would indicate that the olive and the walnut may not be exceptional in their ability to reduce the soil moisture to the hygroscopic point at the approach of their normal dormant period.

This condition may be considered common in fruit orchards in the semi-arid sections where the rains mainly occur during the dormant period. The reduction of the soil moisture to the hygroscopic point by tree roots apparently is not accompanied by a marked harmful effect, provided it is not prolonged too far into the winter period. It seems quite probable that the soil moisture between the wilting point and the hygroscopic point is of vital importance for maintenance of life and the ripening processes of tree growth.

FALL GROWTH OF WALNUT TREES IN RELATION TO DIFFERENCES IN  
SOIL MOISTURE

The length of the growing season of the walnut tree may be somewhat prolonged in the late summer and early fall by the presence of an abundance of soil moisture, and, vice versa, it may be shortened by a lack of sufficient soil moisture. The effect of late irrigation in prolonging the growth of the trees has been so often observed that it need not be dwelt upon at length in this paper; suffice it to say that it is often associated with injury to the trees by the first fall frosts, and for this reason alone the late irrigation of young walnut trees is inadvisable.

TABLE 9  
SOIL MOISTURE IN PEACH AND APRICOT ORCHARDS  
Three-year-old peach orchard, December 1, 1921. (Trees nearly dormant,  
three-quarters defoliated.)

Ft.	Hygroscopic coefficient	Moisture observed	Ratio
1.....	4.91	2.98	0.61
2.....	5.42	4.81	0.89
3.....	6.16	5.84	0.95
Mean	—	—	0.82

Six-year-old apricot orchard, December 1, 1921. (Trees partly dormant,  
one-half defoliated, leaves yellowish green.)

Ft.	Hygroscopic coefficient	Moisture observed	Ratio
2.....	4.96	3.82	0.77
2.....	5.86	4.61	0.79
2.....	5.33	4.37	0.82
2.....	5.84	4.70	0.80
2.....	4.85	4.26	0.88
2.....	5.69	4.38	0.77
2.....	5.58	4.80	0.86
2.....	5.61	4.78	0.85
2.....	5.16	4.48	0.87
2.....	4.42	4.38	0.99
2.....	4.88	4.71	0.97
2.....	5.08	4.85	0.96
Mean			0.86



It has been of especial interest in this investigation to determine the dryness of the soil necessary to entirely check the growth of the tree and thus promote the commencement of the dormant period. The year 1921 was favorable for this sort of observation, inasmuch as the preceding winter rains were below the average and penetrated to a depth of only about 37 inches. With the addition of water from winter irrigation (see table 4) the depth of moist soil on plots A and C was not more than 6 feet. The water level in this grove is about 80 feet from the surface, and the soil below the reach of the rains or irrigation is constantly at a point of extreme dryness. Thus the lower root zone may remain at the hygroscopic point or slightly below this point for twelve to eighteen months at a time during seasons of scanty rainfall. Since summer irrigation did not penetrate more than 7 feet, it is reasonably certain that there was no soil moisture above the hygroscopic point in the lower root zone of the plots in question during the entire growing season of 1921. The greatest amount of water applied at any one time was 4.2 acre inches per acre, with twenty days as the shortest interval between irrigations.

Plots A and C were irrigated for the last time on August 9 and July 19, respectively. On September 13 it was noted that the trees on plot C were maturing somewhat faster than those on plot A. The foliage of the trees of plot C was beginning to turn yellowish green, especially the leaves in the centers of the trees, and a few leaves were beginning to collect on the ground. At the same time the foliage of the trees on plot A was still green. The twig growth had terminated on both groups of trees. None of the trees appeared wilted, although the maximum temperature at 2 P.M. was 85° F.; the relative humidity was 35 per cent, and the soil moisture was reduced to practically the hygroscopic point as shown in table 10.

The above observation was made 34 days after A was irrigated and 54 days after C was irrigated, both plots receiving 4.2 acre inches per acre. Since the same degree of dryness of the soil had been reached in the two plots, the difference in maturity of the trees may be taken to indicate that a prolonged period with the soil moisture between the wilting point and the hygroscopic point is necessary to check the growth of the walnut tree during the warm months of early fall.

The statement is made in a preceding paragraph that, "the soil below the reach of the rains or irrigation is constantly at a point of



extreme dryness.” It is of interest at this point to note observations which were made in this regard on November 17, 1922. A hole was bored 20 feet deep with a post hole auger, and each foot-level was kept separate for moisture observations. The following summary shows the relative moistness of the soil.

Foot-level	Hygroscopic point	Ratio of moisture observed to hygroscopic point
1	3.2	2.9
2	3.0	1.4
3	3.6	0.9
4	4.2	0.9
5	4.7	0.9
6	4.9	0.9
7	5.5	0.7
8	3.1	1.1
9	2.6	1.1
10	4.3	0.9
11	2.8	0.8
12	1.7	1.5
13	2.6	1.2
14	1.3	1.1
15	5.4	1.1
16	6.8	1.0
17	4.2	1.3
18	5.9	0.9
19	4.8	0.9
20	4.4	0.9
Mean (eliminating 1st foot)	4.0	1.0

Except for the surface foot which had been wet by recent rains, and a sand layer in the twelfth foot, the moisture was very close to the hygroscopic point or below it.

TABLE 10

SUMMARY OF THE SOIL-MOISTURE OBSERVATIONS FOR PLOTS A AND C, SEPTEMBER 13, 1921, EXPRESSED AS A RATIO OF THE HYGROSCOPIC POINT

	Plot A	Plot C
Average of 1st ft.....	0.91	0.72
Average of 2nd ft.....	0.98	0.91
Average of 3rd ft.....	0.98	1.00
Average of 4th ft.....	0.93	1.14
Average of 5th ft.....	1.02	0.91
Average of 6th ft.....	1.01	1.05
Average of 7th ft.....	1.06	1.11
Mean	0.98	0.98

## SUMMARY

1. The rainfall of the Hemet Valley is less effective in raising the soil-moisture content of the subsoil than might be thought from a mere statement of total rainfall. Much of the rain falls in small showers of only a few tenths of an inch and is rapidly lost by surface evaporation. For a three-year period the total depth of penetration of moisture averaged 5.2 inches per inch of rainfall during the rainy season.

2. Studies made upon the moisture content of the soil in a walnut grove have shown that at the end of the growing season the moisture was reduced to a point near the hygroscopic coefficient, in spite of summer irrigations totaling 12.5 acre inches for the season. After a period of 169 days without a rain of .3 of an inch or more the moisture in the upper five feet of this soil varied from .54 to .85 of the hygroscopic coefficient. The moisture content of a different type of soil in a three-year-old peach orchard and in a six-year-old apricot orchard showed a similar degree of dryness at the same state in the growth cycle of the trees. The average moisture content in the upper three feet of the former soil was .82 of the hygroscopic coefficient, and in the second foot of the latter was .86 of the hygroscopic coefficient.

3. In spite of the low moisture content of the soil in the latter part of the growing season, the trees showed no permanent wilting but continued to mature and entered the dormant period without apparent injury. Temporary wilting occurred only during the middle of the day when a high temperature was accompanied by a low humidity.

4. The moisture content of the soil was generally at the hygroscopic point at the end of the growing season whatever the amount of water present at the beginning. In other words there was no residuum of water for the use of the trees in the following season.

5. The moisture content of the upper seven feet of the soil in this grove gradually increased with the winter rainfall and usually reached a maximum percentage in March, when the moisture present equaled from 2.5 to 3.5 times the hygroscopic coefficient. Early in April the amount of soil moisture was reduced as the trees started their spring growth. The soil moisture of the upper four feet was approximately at the wilting point by the middle of June. The residual moisture from heavy winter irrigation persisted in the subsoil area until the

middle of the growing season, but by the end it had been gradually taken out of this area, where the tree roots are less numerous. Summer irrigations of 4.2 acre inches per acre raised the water content for a brief time, but within thirty days it dropped back to the approximate value of the wilting point. Such irrigation had little effect on the water content of the soil below the fourth foot. By the end of October the soil in the upper seven feet was approximately at the hygroscopic point.

6. In stating the moisture content of the soil in relation to any of the various conventional coefficients, such as the moisture equivalent, the wilting point, or the hygroscopic coefficient, the field studies with walnuts have shown that the hygroscopic coefficient is the most logical point upon which to base all calculations. If the actual moisture present is stated in comparison with the wilting point, for example, we shall be dealing with minus quantities much of the time. It has seemed to the authors that statement of the moisture in a soil as a ratio of the hygroscopic coefficient gives the most comprehensive conception of the relative moistness. When such a ratio is accompanied by a statement of the hygroscopic coefficient, the type of soil worked with can be clearly understood by the reader.

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STUDIES ON THE EFFECTS OF SODIUM,  
POTASSIUM, AND CALCIUM ON  
YOUNG ORANGE TREES

BY

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STUDIES ON THE EFFECTS OF SODIUM,  
POTASSIUM, AND CALCIUM ON  
YOUNG ORANGE TREES\*

BY

H. S. REED AND A. R. C. HAAS

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EXPERIMENTAL METHODS

It has been pointed out in a previous paper<sup>1</sup> that certain pathological conditions of citrus trees may be due to an excess of sodium salts. Special attention was given in that study to the changes in the growth and composition of young orange trees when they were grown in sand cultures receiving large additions of sodium chlorid with or without the presence of calcium. As the effects produced by sodium salts upon citrus trees are no doubt in a large measure dependent upon the nature of the anions present, it is essential that the effects of sodium salts other than the chlorid be also considered. The present paper deals with the effects produced by the application of solutions containing considerable sodium sulfate to young orange trees grown in sand cultures, and further with the changes brought about in young orange trees when sodium is substituted for potassium in Hoagland's nutrient solution.<sup>2</sup>

Valencia orange (*Citrus sinensis*) trees budded on sour orange (*Citrus aurantium*) rootstocks were used in the experiments. They were planted on May 21, 1920. The details of the culture and of the removal and preparation of the trees for analysis have been previously described.<sup>2</sup>

Trees 1 and 2 received Hoagland's nutrient solution in sand culture.

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\* Paper No. 106, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

The trees in cans 18-23, inclusive, received Hoagland's nutrient solution in which potassium had been substituted for calcium, plus 1000 p.p.m. sodium sulfate.

The trees in cans 24-29, inclusive, received the same culture solution as cans 18-23, plus the calcium of Hoagland's solution added in the form of calcium chlorid.

The trees in cans 47-51, inclusive, received Hoagland's nutrient solution in which sodium had been substituted for potassium.

The concentrations of the various ions in the culture solutions are given in table 1. Approximately 5 to 10 p.p.m. of iron in the form of ferric tartrate were added to distilled water or culture solution prior to their addition to the sand cultures. The concentration of Fe in the culture solution has been called 1 p.p.m., as the amount used was arbitrary, and, as a later paper will point out, it is difficult to keep appreciable amounts of iron in solution in such a nutrient medium.

The culture solutions were distinctly acid in reaction when freshly prepared, but became less so after standing in contact with pure silica sand. Table 2 shows that the change in reaction of the culture solutions was greatest when they were allowed to percolate through sand in which the trees were growing. The pH values of the sap of mature leaves were approximately the same in series 18-23 and 24-29, although in series 47-51 the sap was somewhat more acid in reaction.

Table 3 shows the osmotic pressures of the culture solutions employed, and makes it evident that the pressures were not reflected in the concentration of the leaf sap.

Samples of drainage water from the cans were examined by A. B. Cummins, who found that in each case the percolates contained abundant amounts of  $\text{NO}_3$  and  $\text{PO}_4$ .

TABLE 1

COMPOSITION OF CULTURE SOLUTIONS

Parts per million

	Trees 1 and 2	Trees 18-23	Trees 24-29	Trees 47-51
Fe.....	1	1	1	1
Mn.....	0.1	0.1	0.1	0.1
Ca.....	159	0.0	159	159
Mg.....	54	54	54	54
K.....	185	496	496	0.0
Na.....	7	330	330	115
PO <sub>4</sub> .....	105	105	105	105
SO <sub>4</sub> .....	216	890	890	214
Cl.....	10	10	291	10
NO <sub>3</sub> .....	718	718	718	718
Total concentration.....	1455.1	2604.1	3044.1	1376.1
Salts employed in making the nutrient solution.....	KNO <sub>3</sub> MgSO <sub>4</sub> NaCl Ca(NO <sub>3</sub> ) <sub>2</sub> KH <sub>2</sub> PO <sub>4</sub> MnSO <sub>4</sub>	KNO <sub>3</sub> MgSO <sub>4</sub> NaCl KH <sub>2</sub> PO <sub>4</sub> Na <sub>2</sub> SO <sub>4</sub> MnSO <sub>4</sub>	KNO <sub>3</sub> MgSO <sub>4</sub> NaCl KH <sub>2</sub> PO <sub>4</sub> Na <sub>2</sub> SO <sub>4</sub> CaCl <sub>2</sub> MnSO <sub>4</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub> NaNO <sub>3</sub> MgSO <sub>4</sub> NaCl NaH <sub>2</sub> PO <sub>4</sub> MnSO <sub>4</sub>

TABLE 2

INITIAL AND FINAL pH VALUES OF THE CULTURE SOLUTIONS

Series	Initial pH of culture solution	pH of culture solution after 4 weeks			pH of sap of mature leaves
		Without sand	* In contact with sand	Sand and tree roots	
1 and 2	5.2	5.2	5.5	6.8	6.00
18-23	5.3	5.4	6.7	7.0	5.85
24-29	5.2	5.3	6.1	6.8	5.87
47-51	5.2	5.3	6.1	6.6	5.77

TABLE 3  
OSMOTIC PRESSURES OF CULTURE SOLUTIONS AND OF SAP OF MATURE  
ORANGE LEAVES

Series	Osmotic pressure	
	Culture solution (atm.)	Leaf sap (atm.)
1 and 2	0.728	20.80
18-23	1.001	23.10
24-29	1.218	20.45
47-51	0.531	21.89

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF TREES  
GROWN IN THE DIFFERENT CULTURES

In the series 18-23, where the trees were grown in a solution minus calcium but plus sodium sulfate, there were marked symptoms of injury, quite similar to those of series 6-11 and 42-46 previously described,<sup>1</sup> namely, abundant abscission of leaves, tendency for the leaves to mottle, peppering of leaves with brown dead spots, abnormal curling of leaves, dying of shoot tips, multiple bud formation, and dying of the upper end of trunks. The trees were in very poor condition and it seemed unlikely that they would grow much longer, as the new growth they had produced was very short-lived. On May 26, 1921, the upper half of each tree in series 18-23 was removed. The condition of the remaining portion was only temporarily improved. Plate 1, figure 1, shows the effect of the repeated abscissions and the pushing out of new foliage after each abscission. On September 20, 1921, trees 18-23 were removed from the containers. The roots in most cases were badly stunted and many of the rootlets were slimy and gelatinous. Plate 1, figure 2, shows the root systems in this series as contrasted with those produced during the same time in sand which received Hoagland's nutrient solution.

In series 24-29, where both calcium and sodium sulfate were present, trees 25 and 29 failed to make a satisfactory start and were therefore removed and new trees planted in their places. Tree 26 was quite chlorotic and stunted in appearance, and although CO<sub>2</sub> and iron solution were added to the particular sand culture used in this

case, its appearance did not improve. When the tree was removed from the can it was found that the primary root was rotted practically throughout its entire length. Trees 26, 27, and 28 were removed from their cans on September 20, 1921, while trees 24, 25, and 29 were not removed until February 10, 1922. The trees of this series, tree 26 excepted, were excellent specimens with dark green leaves and healthy vigorous root systems. Plate 2, figure 1, shows the condition of tree 24 in September, 1921. It was quite similar to that of trees 27 and 28. Obviously only trees 24, 27, and 28 are comparable in growth and composition with tree 1 produced in Hoagland's solution. As trees 25 and 29 were replants and therefore of a different age, we cannot expect them to be the same in composition and size as the other trees in the series.

Plate 2, figure 2, shows the excellent root systems of trees 27 and 28 as compared with the growth made in sand receiving Hoagland's nutrient solution (tree 1). In 1920, a considerable number of mature leaves fell from trees 24, 27, and 28, during late summer, which was not the case with trees grown in Hoagland's solution.

Trees 24, 25, and 29 were left growing until February, 1922, in order to determine whether mottling would occur. No mottling developed in any of the trees of this series, to which calcium was liberally supplied. The absence of mottling in this series, where 1000 p.p.m. sodium sulfate was added to the culture solution, as well as in series 12-17, where 1000 p.p.m. sodium chlorid was added, make it extremely doubtful whether mottling is primarily due to an excess of sodium salts in the nutrient medium. The literature bearing upon the effects of sodium salts on plants has been discussed in the previous paper.<sup>1</sup>

The tops of the trees in series 47-51, where potassium was omitted from the culture solution, were all quite large and vigorous. Tree 51 was rather small when it was planted, but was very thrifty as growth progressed. Plate 3 shows the condition of a typical tree in September, 1921, when trees 48 and 50 were removed. The remaining trees were left growing until February 15, 1922, but no further changes were observed during the additional period of growth.

The root systems of trees 47-51 compare very favorably with the growth obtained when Hoagland's nutrient solution was used (tree 1). Plate 4, figure 1, shows the root systems of trees 1 and 48.

Some of the leaves of trees 47-51 were undersized and the chlorophyll in many of the leaves in this series tended to fade out, as is seen in plate 4, figure 2. The bronze-yellow coloration thus produced was usually greatest on the side of the tree longest exposed to the direct rays of the sun. The absence of potassium was accompanied by localized dead areas, usually on one side of the leaf. In the more advanced stages of injury the dead areas on the leaves dried out and were traversed by fissures, as is shown in the lower row of plate 5, figure 1. The new leaves produced after the lack of potassium became apparent were at first light green in color with the venation a darker green, so that the veins were very distinct and conspicuous (pl. 5, fig. 2). As the leaves matured, they ultimately acquired a uniform dark green color. In contrast with the trees in the calcium-free series, 6-11, 18-23, and 42-46, in which the leaves readily abscised, we find that in the potassium-free series the leaves are held firmly attached for a considerable time after changing color.

The bronze-yellow coloration of the leaves and the destruction of the chlorophyll were due no doubt to the deficiency of potassium in the culture solution.

The dry weights of the various portions of the trees and their water requirement are given in table 4.

It will be seen that the water requirements of trees 27 and 28, and of 48 and 50, which were all in excellent condition when removed from the sand cultures, were considerably below those of trees 18-23, which showed considerable injury. (See table 4.) This water relation in plants has been observed by several investigators. The most recent of them were Kiesselbach<sup>10</sup> and Noyes,<sup>11</sup> who have concluded that when the plant is grown in a soil which responds to fertilization there is a consequent decrease in water requirement. This reduction in water requirement per unit of dry matter is due to a more vigorous growth resulting from a more favorable supply of food materials.



TABLE 4  
NUMBER OF LEAVES, DRY WEIGHT OF VARIOUS PORTIONS OF CITRUS TREES, AND WATER TRANSPIRED

No. of tree	No. of leaves on tree	Dry weight (60-65°C) in grams						Total culture solution added Liters	Total distilled water added Liters	Total drainage water Liters	Transpiration Liters	Ratio of total transpiration to total dry weight of tree
		Leaves	Shoots	Trunk	Root	Rootlets	Oranges					
18.....	71	18.2	15.0	69.0	69.5	29.5	.....	181	67	125.9	122.1	607
19.....	42	11.0	9.7	65.0	70.0	15.0	.....	166	50	141.2	74.8	438
20.....	62	24.0	27.0	106.0	172.0	29.5	.....	192	56	122.4	125.6	350
21.....	25	12.0	11.0	40.0	79.5	18.5	.....	169	44	136.3	76.7	476
22.....	66	8.0	14.0	88.0	133.0	22.0	.....	186	50	141.2	94.8	358
23.....	50	14.7	11.0	83.0	120.0	16.0	.....	169	53	142.9	79.1	323
Av. per tree.....	53	14.7	14.6	75.2	107.3	21.8	.....	177.1	53.3	135.0	95.5	425
24.....	686	103.0	62.5	138.0	106.5	123.0	17.0	217	124	172.9	168.1	306
25.....	448	73.0	34.0	120.0	91.5	79.0	.....	169	112	167.7	113.3	285
26.....	331	41.5	25.0	90.0	60.0	23.0	.....	171	46.6	131.9	85.7	358
27.....	843	183.0	89.0	127.0	122.0	142.0	.....	200	133.5	138.0	195.5	295
28.....	655	163.0	84.0	151.0	143.0	106.0	.....	209	109.5	147.9	170.6	264
29.....	271	64.0	20.0	153.0	86.5	29.5	.....	176	84.0	154.5	105.5	299
Av. of trees 27 and 28.....	749	173.0	86.5	139.0	132.5	124.0	.....	204.5	121.5	143.0	183.1	280
Av. per tree for all trees of series 24-29.....	539	104.6	52.4	129.8	101.6	83.8	.....	190.3	101.6	152.5	139.8	301
47.....	737	136.0	64.0	138.0	104.0	160.0	.....	220	135.0	171.9	183.1	304
48.....	649	129.0	53.0	95.0	145.0	112.0	.....	197	90.0	123.3	163.7	307
49.....	721	114.5	66.0	186.0	181.0	145.5	.....	220	132.0	140.8	211.2	305
50.....	850	179.3	55.0	136.0	116.0	93.0	20.0	201	92.0	116.7	176.3	294
51.....	500	89.0	40.0	89.5	115.5	91.0	.....	196	125.0	211.1	109.9	259
Av. of trees 48 and 50.....	750	154.2	54.0	115.5	130.5	102.5	.....	199	91	120.0	170.0	301
Av. per tree for all trees of series 47-51.....	691	129.6	55.6	128.9	132.3	120.3	.....	206.8	114.8	152.8	168.8	294

TABLE 5  
COMPOSITION OF ORANGE TREES GROWN IN MODIFIED NUTRIENT SOLUTION EXPRESSED AS A PERCENTAGE OF DRY MATTER  
(For composition of solutions furnished to different trees see table 1)

No. of Tree	Leaves			Shoots			Trunk			Root			Rootlets (calculated to a silica-free basis)		
	18-23	27 and 28	48 and 50	18-23	27 and 28	48 and 50	18-23	27 and 28	48 and 50	18-23	27 and 28	48 and 50	18-23	27 and 28	48 and 50
Ash	14.82	15.15	13.49	7.21	6.27	5.21	3.06	2.59	2.34	2.61	2.29	2.23	7.08	9.10	7.90
N	2.90	2.92	3.39	1.57	1.30	1.36	0.83	0.74	0.83	0.83	0.84	0.81	2.24	2.21	2.39
S	0.24	0.42	0.32	.....	0.14	0.12	.....	0.06	0.06	0.06	0.08	0.07	0.45	0.65	0.51
P	0.20	0.23	0.27	0.18	0.19	0.26	0.04	0.08	0.11	0.04	0.07	0.10	0.16	0.59	0.90
K	6.76	5.64	0.19	2.97	1.58	0.09	0.37	0.51	0.05	0.33	0.48	0.14	2.11	2.65	0.27
Na	0.45	0.38	0.30	0.25	0.14	0.15	0.31	0.19	0.21	0.33	0.24	0.24	0.61	0.45	0.95
Ca	0.15	1.12	4.08	0.21	0.92	1.24	0.52	0.43	0.62	0.41	0.35	0.46	0.44	0.92	1.77
Mg	0.25	0.15	0.56	0.18	0.14	0.31	0.10	0.05	0.07	0.10	0.06	0.05	0.37	0.17	0.29
Cl	0.03	0.27	0.02	0.02	0.23	trace	trace	0.08	trace	trace	0.07	trace	0.11	0.81	0.13

TABLE 6  
COMPOSITION OF ORANGE TREES GROWN IN MODIFIED NUTRIENT SOLUTION EXPRESSED AS A PERCENTAGE OF THE ASH  
(For composition of solutions furnished to different trees see table 1)

No. of Tree	Leaves			Shoots			Trunk			Root			Rootlets (calculated to a silica-free basis)		
	18-23	27 and 28	48 and 50	18-23	27 and 28	48 and 50	18-23	27 and 28	48 and 50	18-23	27 and 28	48 and 50	18-23	27 and 28	48 and 50
K	45.58	37.21	1.42	41.22	25.17	1.63	11.81	19.60	1.83	12.55	20.85	6.32	29.90	29.12	3.47
Na	3.00	2.49	2.18	3.39	2.15	2.79	9.85	7.18	9.01	12.63	10.25	10.68	8.62	4.91	11.96
Ca	1.03	7.35	30.21	2.87	14.65	23.81	16.99	16.38	26.52	15.65	15.17	20.25	6.21	10.09	22.38
Mg	1.69	0.97	4.17	2.50	2.16	5.95	3.25	2.10	2.77	3.66	2.43	2.11	5.20	1.80	3.67
Cl	0.22	1.80	0.16	0.22	3.61	trace	trace	2.83	trace	trace	2.69	trace	1.60	8.87	1.60
SO <sub>4</sub>	3.76	5.57	4.50	3.00	4.41	5.77	2.58	3.47	4.34	3.43	3.06	3.77	19.04	14.61	18.54
PO <sub>4</sub>	4.12	4.01	5.12	6.40	6.03	8.89	4.08	8.66	13.80	4.39	9.24	13.41	5.05	17.00	23.69

## DATA ON THE COMPOSITION OF THE TREES

A chemical study was made only of the trees which were removed from the cultures in September, 1921, the object of the experiment being to compare trees which had been growing for the same length of time. The trees were prepared for analysis in a manner similar to that previously employed, that is, the finely ground plant materials were composited for the various portions of the trees, as heretofore, and analyzed. The analytical data are presented in tables 5 and 6.

The total ash when expressed as percentage of dry matter amounted to approximately 14.5 per cent of the leaves, 6 per cent of the shoots, 2.5 per cent of the trunks and roots, and 8 per cent of the rootlets.

The percentage of total nitrogen varied in different parts of the tree: leaves and rootlets > shoots > trunks and roots. (See table 5.)

If we examine the data in table 7 for the percentages of Na in the ash of the various portions of the trees of the different series, we find that in every case (with the exception of the rootlets of trees 48 and 50) the trunks and roots each contain larger percentages of Na than any other portions of the trees. The percentages of Na in the shoots are not appreciably different from those in the leaves. Upon comparing trees 1 and 2 with the other series it is evident that a great increase in the amount of Na in the culture solution is in general accompanied by an increase, not by any means proportionate, in the percentage of Na found in the various portions of the trees. The absolute amount of Na in the various parts of the trees (table 8) fails to show a close correspondence with the amounts of sodium salt in the culture solutions. Trees 27 and 28 which received 330 p.p.m. Na contained only about twice as much of that ion as trees 1 and 2, which received 7 p.p.m. Na. Trees 48 and 50 contained approximately the same absolute amount of Na as trees 27 and 28, although they received only half the amount of Na in the culture solution as it was applied to them. The larger sizes of trees 1 and 2 offset in part the lower percentage of Na when the absolute content of the ion is calculated from the analysis.

TABLE 7

PERCENTAGE OF SODIUM AND SULFATE IN ASH OF TREES RECEIVING VARIOUS CULTURE SOLUTIONS

	Na				SO <sub>4</sub>			
	Trees 1 and 2	Trees 18-23	Trees 27 and 28	Trees 48 and 50	Trees 1 and 2	Trees 18-23	Trees 27 and 28	Trees 48 and 50
Culture solution contained	7 p.p.m. Na	330 p.p.m. Na	330 p.p.m. Na	115 p.p.m. Na	216 p.p.m. SO <sub>4</sub> 10 p.p.m. Cl	890 p.p.m. SO <sub>4</sub> 10 p.p.m. Cl	890 p.p.m. SO <sub>4</sub> 291 p.p.m. Cl	214 p.p.m. SO <sub>4</sub> 10 p.p.m. Cl
Leaves.....	1.17	3.00	2.49	2.18	3.18	3.76	5.57	4.50
Shoots.....	2.56	3.39	2.15	2.79	2.76	3.00	4.41	5.77
Trunk.....	6.74	9.85	7.18	9.01	2.71	2.58	3.47	4.34
Root.....	3.74	12.63	10.25	10.68	3.49	3.43	3.06	3.77
Rootlets.....	0.92	8.62	4.91	11.96	8.68	19.04	14.61	18.54

TABLE 8

TOTAL AMOUNT IN GRAMS OF SODIUM AND SULFATE FOUND IN VARIOUS PARTS OF AVERAGE TREE

	Trees 1 and 2		Trees 18-23		Trees 27 and 28		Trees 48 and 50	
	Na	SO <sub>4</sub>	Na	SO <sub>4</sub>	Na	SO <sub>4</sub>	Na	SO <sub>4</sub>
Leaves.....	0.2666	0.7213	0.0662	0.0823	0.6574	1.4705	0.4626	0.9406
Shoots.....	0.1432	0.1504	0.0365	0.0321	0.1211	0.2422	0.0810	0.1620
Trunk.....	0.3360	0.1440	0.2331	0.0602	0.2641	0.1251	0.2426	0.1155
Root.....	0.1656	0.1518	0.3541	0.0966	0.3180	0.0928	0.3132	0.1175
Rootlets.....	0.1013	0.9698	0.1330	0.2943	0.5580	1.6492	0.9738	1.5068
Total per average tree	1.0127	2.1373	0.8229	0.5655	1.9186	3.5798	2.0732	2.8424

TABLE 9

TOTAL AMOUNTS OF Na AND SO<sub>4</sub> IN TREES IN TERMS OF THEIR REACTION VALUES

	Trees 1 and 2		Trees 18-23		Trees 27 and 28		Trees 48 and 50	
	Na	SO <sub>4</sub>	Na	SO <sub>4</sub>	Na	SO <sub>4</sub>	Na	SO <sub>4</sub>
Leaves.....	.01157	.01500	.00287	.00171	.02853	.03059	.02008	.01956
Shoots.....	.00621	.00313	.00158	.00067	.00526	.00504	.00352	.00337
Trunk.....	.01458	.00300	.01012	.00125	.01146	.00260	.01053	.00240
Root.....	.00719	.00316	.01537	.00201	.01380	.00193	.01359	.00244
Rootlets.....	.00440	.02017	.00577	.00612	.02422	.03430	.04226	.03134
Totals.....	.04395	.04446	.03571	.01176	.08327	.07446	.08998	.05911



Reference to table 9 shows the equivalent amounts of Na and  $\text{SO}_4$  in the trees. These calculations were based upon the "reaction values" of the ions under discussion. The reaction values are computed from the formula

$$\frac{V}{W} C = \text{the reaction value,}$$

where  $V$  = the valence of the ion,  $W$  = the atomic weight, and  $C$  = the concentration. It appears that in the case of trees receiving full nutrient solution (trees 1 and 2) and of trees receiving full nutrient plus sodium sulfate (trees 27 and 28), the totals of Na and of  $\text{SO}_4$  are nearly equivalent, but that there is considerable disparity in the case of the other trees. Where the disparity exists there is always more Na than  $\text{SO}_4$  in the tree.

It seems reasonable to assume that the trees growing in the modified solutions did not absorb  $\text{SO}_4$  in amounts equivalent to the Na taken up, and that other anions were taken to balance the Na.

Table 7 shows that an increase in the concentration of  $\text{SO}_4$  in the culture solution may not cause any appreciable increase in the percentage of  $\text{SO}_4$  in the ash of the various parts of the trees. Trees 18-23 and 27 and 28 received a culture solution containing more than four times the  $\text{SO}_4$  concentration which trees 48 and 50 received, and still, in general, we find the percentages of  $\text{SO}_4$  in the various portions of trees 48 and 50 to be slightly greater than those in the corresponding portions of trees 27 and 28. This is due, no doubt, to the Cl content of the culture solution received by trees 27 and 28, as is seen in table 10.

TABLE 10  
PERCENTAGE OF CHLORINE IN ASH OF TREES RECEIVING VARIOUS  
CULTURE SOLUTIONS

	Trees 1 and 2	Trees 18-23	Trees 27 and 28	Trees 48 and 50
Culture solution contained	10 p.p.m. Cl	10 p.p.m. Cl	291 p.p.m. Cl	10 p.p.m. Cl
Leaves.....	0.29	0.22	1.80	0.16
Shoots.....	0.13	0.22	3.61	Trace
Trunk.....	0.02	Trace	2.83	Trace
Root.....	0.13	Trace	2.69	Trace
Rootlets.....	1.01	1.60	8.87	1.60

Table 10 shows the marked increase in the percentage of Cl in the ash of the various portions of trees 27 and 28, cases in which the Cl content of the culture solution was increased. Further investigations will be necessary to determine the absorption of SO<sub>4</sub> and Cl when the trees receive culture solutions containing large, as well as equimolecular, concentrations of SO<sub>4</sub> and Cl.

TABLE 11  
DISTRIBUTION OF POTASSIUM IN TREES (EXPRESSED IN PERCENTAGE OF ASH)

Culture solution contained	Trees 1 and 2	Trees 18-23	Trees 27 and 28	Trees 48 and 50
	7 p.p.m. Na 185 p.p.m. K	330 p.p.m. Na 496 p.p.m. K	330 p.p.m. Na 496 p.p.m. K	115 p.p.m. Na 0 p.p.m. K
Leaves.....	24.73	45.58	37.21	1.42
Shoots.....	16.04	41.22	25.17	1.63
Trunk.....	11.47	11.81	19.60	1.83
Root.....	11.15	12.55	20.85	6.32
Rootlets.....	18.57	29.90	29.12	3.47

Table 11 shows the distribution of potassium in the trees of the several series. A comparison of the percentages of Na and of K in the ash (tables 7 and 11) shows that the ash of orange trees may contain very considerable amounts of K when the roots are in contact with a plentiful supply of this element, or very small amounts when K is omitted from the culture solution. It is extremely interesting to find the leaves of trees 48 and 50 in such excellent condition when they contain less than 2 per cent of K in their ash. The Na content of the culture solution of trees 48 and 50 was half that of trees 18-23 and 27 and 28. The percentages of Na in the ash of the various portions of trees 48 and 50 were in general somewhat greater than the values for trees 27 and 28. It appears, therefore, that the percentages of Na in the ash of the various portions of trees 48 and 50 were somewhat increased when K was omitted from the culture solution. In the nutrition of orange trees no evidence exists to prove that Na performs any of the functions of K in the tree.

It is of interest in this connection that Hartwell, Wheeler and Pember<sup>12</sup> held a contrary opinion. They reported that although Na seemed to cause no increase in growth when an optimum amount of K was present, yet when there was a deficiency of K great enough to cause a 30 per cent depression in the green weight of wheat plants, the addition of Na did increase growth, usually to the amount of 10 per cent or more. A substitution of Ca for Na did not increase growth either with an optimum amount or a deficiency of K. When seedlings were grown a larger amount of K was left in the solution where K was supplemented by Na. Pfeiffer, Rippel, and Pfoten-hauer<sup>13</sup> also believed that Na was used to some extent as a substitute for K by the leaves and stems of oat plants.

Table 12 shows the distribution of Ca in the trees. In trees 1 and 2, 18-23, and 27 and 28, the trunks and roots contained higher percentages of Ca in their ash than the other parts of the trees. The percentages of Ca in the trunks and roots of trees 18-23 and 27 and 28 agree very closely. It is of interest to note the high percentages of Ca in the ash of the various portions of trees 48 and 50, and the relatively reduced percentages for trees 27 and 28 when compared with the percentages for trees 1 and 2, in view of the fact that all three series received the same concentration of Ca in their culture solutions.

Trees 47-51 retained their mature leaves exceptionally well, some effort being necessary in order to remove them.

TABLE 12

DISTRIBUTION OF CALCIUM IN TREES (EXPRESSED IN PERCENTAGE OF ASH)

	Trees 1 and 2	Trees 18-23	Trees 27 and 28	Trees 48 and 50
Culture solutions contained	159 p.p.m. Ca 7 p.p.m. Na 185 p.p.m. K	0 p.p.m. Ca 330 p.p.m. Na 496 p.p.m. K	159 p.p.m. Ca 330 p.p.m. Na 496 p.p.m. K	159 p.p.m. Ca 115 p.p.m. Na 0 p.p.m. K
Leaves.....	18.71	1.03	7.35	30.21
Shoots.....	21.98	2.87	14.65	23.81
Trunk.....	24.34	16.99	16.38	26.52
Root.....	23.42	15.65	15.17	20.25
Rootlets.....	18.88	6.21	10.09	22.38

Ehrenberg<sup>14</sup> has found that if a plant is poorly supplied with K and is liberally supplied with Ca, there results a decreased absorption of K, accompanied by effects detrimental to the plants. The injuries are apparently not so much due to the increased absorption of Ca as to the decreased absorption of K, for it is claimed that by increasing the K content of the culture medium the injurious effects are greatly ameliorated.

Table 13 shows that in the absence of Ca (series 18-23) or in the presence of an increased content of Na, SO<sub>4</sub>, and Cl in the culture solution (series 24-29), no significant differences occurred in the percentages of Mg in the ash of the various portions of the trees when compared with corresponding portions of trees 1 and 2 (except in the rootlets of trees 18-23 and 27 and 28, and the leaves of trees 27 and 28). The various portions of trees 18-23 showed slightly greater percentages of Mg in their ash than the corresponding portions of trees 27 and 28. This increase of Mg in the ash when Ca is omitted from the culture solution is, however, very small and of doubtful significance. The leaves and shoots of trees 48 and 50 show unusually large percentages of Mg in their ash when compared with the same portions in other series, or with the values obtained by Kelley and Cummins<sup>15</sup> for leaves gathered in the field.

TABLE 13  
DISTRIBUTION OF MAGNESIUM IN TREES (EXPRESSED IN PERCENTAGE OF ASH)

Culture solution contained	Trees 1 and 2	Trees 18-23	Trees 27 and 28	Trees 48 and 50
	159 p.p.m. Ca 54 p.p.m. Mg	0 p.p.m. Ca 54 p.p.m. Mg	159 p.p.m. Ca 54 p.p.m. Mg	159 p.p.m. Ca 54 p.p.m. Mg
Leaves.....	1.95	1.69	0.97	4.17
Shoots.....	2.87	2.50	2.16	5.95
Trunk.....	2.95	3.25	2.10	2.77
Root.....	2.78	3.66	2.43	2.11
Rootlets.....	3.02	5.20	1.80	3.67

TABLE 14  
DISTRIBUTION OF PO<sub>4</sub> IN TREES (EXPRESSED IN PERCENTAGE OF ASH)

Culture solution contained	Trees 1 and 2	Trees 18-23	Trees 27 and 28	Trees 48 and 50
	105 p.p.m. PO <sub>4</sub>	105 p.p.m. PO <sub>4</sub>	105 p.p.m. PO <sub>4</sub>	105 p.p.m. PO <sub>4</sub>
Leaves.....	3.97	4.12	4.01	5.12
Shoots.....	8.11	6.40	6.03	8.89
Trunk.....	4.41	4.08	8.66	13.80
Root.....	8.69	4.39	9.24	13.41
Rootlets.....	17.14	5.05	17.00	23.69

Table 14 shows the distribution of  $\text{PO}_4$  in the ash of the various portions of the trees. The percentage of  $\text{PO}_4$  in the different portions of trees 48 and 50 is considerably higher than that for the corresponding portions of trees in the other series, although all four of the series received culture solutions containing equal concentrations of  $\text{PO}_4$ . The percentages of  $\text{PO}_4$  in the rootlets of the trees in these series appear to be proportionally related to their growth, since a low percentage was found in the poor rootlets of 18-23 and a high percentage in the vigorous rootlets of 48 and 50.

The percentage composition of the ash and the effects upon the growth of the trees would seem to depend not only upon the concentration of a particular ion, but also upon the nature and concentration of other ions present in the culture solution.

## DISCUSSION

The absorption of ions and their distribution in the plant are not simple problems. The results presented in the foregoing pages contradict some of the concepts of our predecessors, who thought that nutrition was governed by such supposedly simple conditions as concentration, "availability," diffusion, and the like. The effects of salts, or of their ions, upon plants seem to be determined by two sets of factors, of which one is environmental, the other hereditary.

Using plants of a certain species, one will find that the effects of a given ion are, to a certain extent, related to the concentration of that ion in the external medium. A plant of another species may, however, show very different responses to equivalent concentrations of the ion. The effect of an ion is doubtless further dependent upon environmental factors such as heat and light, although much is yet to be learned in this respect.

Hereditary, or internal, factors may determine to a large extent the absorption and distribution of ions. Certain plants absorb a given ion much more rapidly than do those of another species growing in the same environment. Again, a change in the concentration of an ion in a given environment is not necessarily reflected in the composition of a plant, although it may produce very specific effects on its functions. In the studies presented here, for example, it is evident that an increase in the amount of sodium added to the cultures is not



proportionally reflected in the analyses of the trees. Furthermore, the presence of calcium alters the effects of a given amount of sodium on the trees. The results of our investigations are such that we must recognize a capacity on the part of the tree to do something which cannot be regarded as a simple process of addition or subtraction. This does not mean that it is necessary as yet to invoke the operation of any vitalistic force, but merely that the plant is a dynamic entity with regulatory and selective processes which must be recognized in studying its reaction to its surroundings.

Returning to the question of immediate concern, we may survey the findings presented in this paper.

The young orange trees in the cultures made good growth when the complete nutrient solution was regularly supplied. The growth was very poor when trees were supplied with a modified solution in which calcium was lacking and sodium was greatly increased. A fair amount of growth was made when a large amount of sodium sulfate was added to the nutrient solution. A comparison of the dry weights of the trees shows approximately the following ratios: full nutrient:high sodium sulfate without calcium salts:high sodium sulfate with calcium salts:: 34:10:20.

The concentrations of the nutrient solutions were quite different in the three cases, in fact the modified solutions were approximately twice the strength of the complete nutrient solution. There is no evidence, however, that these concentrations of themselves were great enough to produce detrimental effects upon the trees. Within the range of concentrations employed the total concentration had less effect upon the tree than changes in the proportions of various salts. This is not surprising when we consider that plants must experience constant fluctuations in the concentration of the soil solution in which they grow and that they are less affected by ordinary fluctuations in concentration than by changing proportions in the salts of the soil solution.

The foliage of trees grown in the modified solutions shows striking effects when compared with that of trees grown in the complete solution. When calcium salts are omitted there is early and frequent abscission of leaves. Succeeding crops of leaves fall while they are still quite immature. A condition is finally reached where the younger parts of the tree are entirely leafless. If a large amount

of sodium is added to a nutrient solution which lacks calcium, the leaves show a tendency to mottle before abscission occurs. The foliage on trees which received large amounts of sodium salts in addition to the complete nutrient solution showed, however, no serious detrimental effects except for premature abscission of a few leaves during the warmest part of the summer. This fact indicates a function of calcium salts which, in the case of orange trees, has not hitherto been appreciated. At the same time it shows the scarcity of information about the relation of sodium and calcium salts to chlorophyll formation.

The results of our experiments indicate that orange trees may function with considerably less potassium than they ordinarily absorb. Trees which are grown in a complete nutrient solution are relatively rich in potassium. The high percentage of potassium in their leaves has frequently been mentioned. Nevertheless the trees which received no potassium salts were able to grow fairly well during the experimental period of seventeen months, subsisting, no doubt, on the potassium that was in them at the time they were planted in the cultures. The low percentages of potassium in the leaves and shoots contrasted strongly with that found in homologous parts of trees in other cultures, yet the functions of these organs seem not to have been so seriously impaired as their ash content might indicate. If the experiment had been continued much longer, there is no doubt that serious injury would have occurred from the prolonged absence of potassium.

Stoklasa and his associates<sup>3</sup> claimed that the emanations from the potassium salts in plants are of great importance to plant life. They are of the opinion that potassium sends out  $\beta$ - and  $\gamma$ -rays which permeate the entire chlorophyll-containing cell and contribute to the production of organic substances by photosynthesis. Loeb,<sup>4</sup> however, finds that the behavior of the potassium ion in antagonistic salt action is due solely to its position in the periodic table or rather to its atomic number, and not to those explosions in its nucleus which give rise to a trace of radioactivity. The conclusions thus far presented are almost entirely speculative in nature and need further experimental support.

The bronzed appearance of the leaves when the culture solution is deficient in potassium is significant in view of the fact that Orton (reported in 5) observed a potato disease which was characterized

by bronzing and later by browning of the leaves of the plant. The primary cause of the disease was considered to be an insufficient potash supply or an excess of nitrates in the presence of a minimum amount of potash.

Molliard<sup>6</sup> found, when all but 1/80 of the potassium phosphate in the normal culture medium was replaced by sodium phosphate, that *Aspergillus niger* still oxidized sugar, but that instead of yielding CO<sub>2</sub> almost entirely, it then formed an important amount of acid also. We might expect the sap obtained from the leaves of trees 47-51 in our experiment, which received a culture solution deficient in potassium, to be more acid than the sap of leaves from series 24-29, which received abundant potassium. Table 2, which shows that the sap of orange leaves from series 47-51 have a greater acidity than that of leaves from series 24-29, confirms this assumption. Molliard further states that when potassium was deficient, the fungus failed to produce the usual black pigment, but formed instead a golden-yellow pigment.

Smith and Butler<sup>7</sup> found that the symptoms of potassium starvation appear early in the life of the plant and consist of a dwarfing of the axis and progressive death of the foliage, the older leaves succumbing first. Morita and Livingston<sup>8</sup> obtained a satisfactory growth of wheat seedlings without potassium for three weeks. Of all the ratios of salts tested, the ones with the lower concentrations of dihydrogen phosphate produced the best plants.

Hoagland<sup>16</sup> found that stems and leaves of barley contain increased amounts of potassium when grown in more concentrated solutions and that, in the dry plant material, the increased amounts are largely soluble in water. Kostychev and Eliasberg<sup>9</sup> found that the potassium of the plants examined could be completely extracted by means of water. On incinerating the extracted residue, the ash was found to be free from potassium. The lead acetate and tannin precipitates were found to contain no potassium. The aqueous extracts before and after incineration contained the same quantities of potassium. They conclude, therefore, that potassium, unlike other indispensable elements, is not present in the plant, to even a partial degree, in combination with organic matter.

The higher calcium content of trees in cultures to which no potassium was furnished is rather interesting, especially since the sodium content of these trees was not higher than it was in those of the sodium sulfate series except in the root systems. It is noteworthy that in the ash the concentration of a divalent cation should have been increased, rather than that of a monovalent cation when potassium was omitted from the culture solution. The ash content of the leaves of these trees was somewhat lower than that in other cultures, but the percentage of calcium in it was very much higher. Aside from the leaves, the calcium content of the ash of other parts of the tree differed but little from that of trees to which the complete nutrient solution was supplied.

#### SUMMARY

1. Young orange trees showed serious injury when grown in cultures in which no calcium salts were supplied. When sodium sulfate was added to cultures which lacked calcium the first leaves had a tendency to be mottled, were abnormally curled, and were shed prematurely. Successive crops of leaves were full of small dead spots and were likewise prematurely shed. The shoots grew poorly and had a tendency to form multiple buds. The roots of such trees made restricted growth.

2. The condition of trees receiving sodium sulfate was much better if calcium salts were also present. The foliage, shoots, and roots showed no detrimental effects from the treatment, though the total amount of growth was less than that of trees receiving a complete nutrient solution.

3. Orange trees in cultures to which no potassium salts were added made a fair growth, and at the end of seventeen months showed no such injury as those which lacked calcium had shown. There was a tendency for the chlorophyll to fade out, but no premature abscission occurred. The sap of the leaves was slightly more acid than that from trees grown in other cultures where they received potassium and sodium salts.

4. Leaves of trees to which no calcium salts were supplied were very rich in potassium, and conversely, those receiving no potassium salts were significantly high in calcium.

5. The trunks and roots contained higher percentages of sodium than other parts of the tree. The increment in sodium in roots and rootlets was especially marked in the case of trees receiving increased amounts of sodium salts.

6. The rootlets were richer in phosphate, sulfate, and chlorid ions than other parts of the trees.

7. Where calcium salts were withheld from the trees the trunks and roots were the last to be depleted of that ion.

8. Where potassium salts were withheld, the roots and rootlets were the last to be depleted of that particular ion.



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## PLATE 1

Fig. 1. Tops of trees in series 18-23 which received Hoagland's solution from which calcium was omitted and to which 1000 p.p.m. of sodium sulfate was added. The upper parts of the trees having died, they were pruned off several months before the picture was taken. The photograph shows the appearance of the trees after sixteen months' treatment.

Fig. 2. Root systems of trees 18 and 22 (grown in Hoagland's solution lacking calcium, plus 1000 p.p.m. of sodium sulfate) compared with roots of tree 1, (grown in Hoagland's solution).



Fig. 1

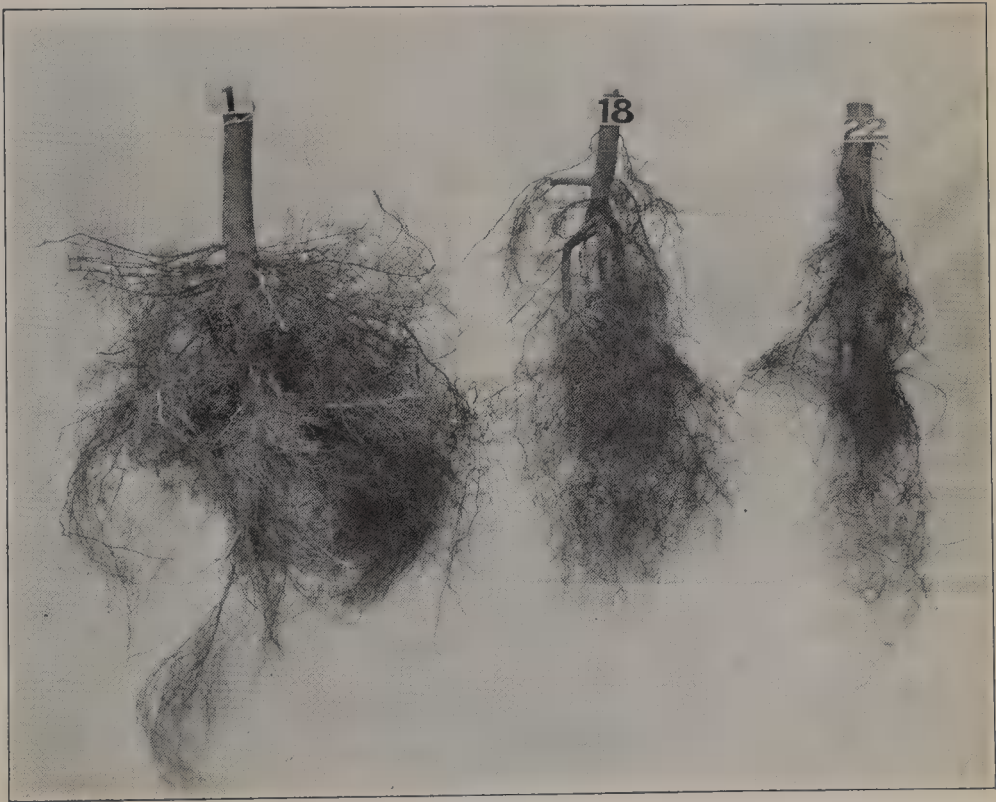


Fig. 2







## PLATE 2

Fig. 1. Top of tree 24 which received the same amounts of potassium and of sodium sulfate as those in series 18-23 with the addition of 159 p.p.m. calcium in the form of calcium chlorid.

Fig. 2. Root systems of trees 27 and 28 (grown in solution with high sodium sulfate content plus calcium) compared with roots of tree 1 (grown in Hoagland's solution).



Fig. 1



Fig. 2





PLATE 3

Top of tree 47 which received Hoagland's solution in which sodium was substituted for potassium. The photograph shows the tree after sixteen months' treatment.









#### PLATE 4

Fig. 1. Root system of tree 48, grown seventeen months in Hoagland's solution in which sodium was substituted for potassium, compared with roots of tree 1, grown in Hoagland's complete solution.

Fig. 2. Leaves from trees 47-51. Lower leaves show initial stages of injury characterized by bronze-colored stripes on either side of the midrib. Upper leaves show advanced stages of injury resulting from potassium deficiency. The center leaf shows a dead spot in the bronze-colored area. The outer leaves show a general destruction of chlorophyll.

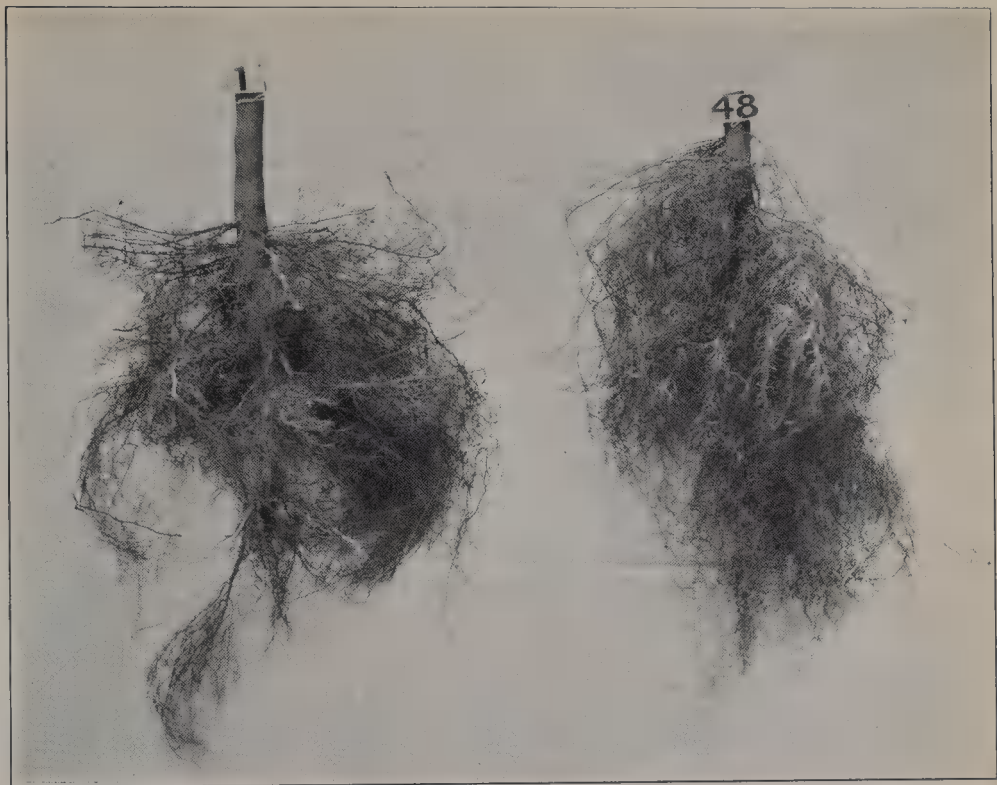


Fig. 1



Fig. 2







## PLATE 5

Fig. 1. Comparison of orange leaves showing different types of injury. Leaves in the upper row from series receiving 1000 p.p.m. of sodium salts in addition to the nutrient solution. The injury consists of small dead spots distributed quite at random. Leaves in the lower row from trees which received no potassium. Their injury consists of a dead strip on one side of the leaf about midway between the margin and the midrib.

Fig. 2. Young leaves from trees 47-51, which show chlorosis although the veins are still green. Subsequently such leaves became dark green throughout.



Fig. 1



Fig. 2



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D. R. HOAGLAND\*

(Contribution from the Division of Plant Nutrition, University of California)

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For several years considerable attention has been given in this laboratory to the study of the absorption of ions by various plants under controlled solution culture conditions. Incidental to this work, numerous observations have been made of the changes in the reaction of culture solutions induced by the growth of various plants, and it is now intended to discuss very briefly certain data relating to this phase of the investigation, and to suggest the bearing which the experiments in question may have on the processes associated with the intake of ions by plants. While the literature pertaining to the relations between hydrogen ion concentration and biological phenomena has now reached enormous proportions, the extension of knowledge in this field is still profitable, since the reaction of the medium may frequently become the most important variable in biological systems, as has been so clearly illustrated, for example, by Loeb<sup>1</sup> in his recent enlightening researches on proteins and colloidal behavior.

In a previous paper<sup>2</sup> attention was called to the fact that ordinary culture solutions containing nitrate, when placed in contact with growing plants, tend to change their reactions very rapidly. If the initial reaction be acid, the pH value of the solution increases

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\*The writer desires to acknowledge the assistance of Mr. D. C. Caudron in certain of the experiments.

and generally attains an approximately constant value close to the neutral point. Subsequent experiments in different laboratories have, on the whole, been in agreement with this finding.<sup>3, 4</sup> While different solutions show a similar tendency in this regard, the rate at which the reaction is altered is necessarily retarded in a solution like Shive's, which possesses a relatively high buffer value associated with its high proportion of phosphate. This has been shown by Jones and Shive<sup>3</sup> in their comparisons of different culture solutions.

Since the concentration of hydrogen ions undergoes a definite change, obviously there must take place a readjustment in the concentrations of certain other ions. The nature of these changes is illustrated by some determinations of the quantities of the different ions removed from culture solutions by barley plants at different stages of growth. Solutions of similar composition were compared, one of an initial pH value of 5.1, the other 6.8. The solutions were changed weekly and the absorption of ions determined for three separate periods of one week each, beginning with the fourth, sixth and eleventh weeks of growth (table 1).

In this experiment, the total equivalents of anions removed from the acid solution very considerably exceeded those of cations. The equivalence of positive and negative ions was maintained in the solution by the formation of  $\text{HCO}_3$  ions. Thus an ion not originally present in appreciable concentration soon appeared in the culture solution in significant quantity as a result of the activities of the plant. This replacement of the anion of a strong acid (chiefly  $\text{NO}_3$ ) by the weak acid anion  $\text{HCO}_3$ , seems to determine in large measure the change of reaction in culture solutions. Such solutions, however, do not ordinarily attain an alkaline reaction (unless the solution is allowed to concentrate too far), since the excretion of carbon dioxide by the plant prevents the formation of normal carbonate. Under these conditions, the approximately constant reaction reached is very similar for quite diverse types of plants. Theron<sup>5</sup> has determined, for example, the following values: Peas, pH 6.7, barley and corn pH 6.8.

In the experiments referred to above, it may also be noted that the absorption of  $\text{Ca}$  and  $\text{PO}_4$  ions was greater from the more acid solution. The sum of the equivalents of positive and negative ions removed was greater from the solution of pH 5.1 than from the solution of pH 6.8. In two periods of growth, approximately equivalent

quantities of anions and cations were removed from the solution having an initial pH value of 6.8.

It may be asked whether the reaction to which the solution is brought by the growth of the plant is, therefore, the most favorable one for growth. No doubt excellent development of many plants is possible at this reaction, but it cannot be asserted that it is necessarily the optimum. With certain plants, for example, barley, cucumbers, peas, an appreciably more acid reaction, continuously maintained in this solution, seems to be accompanied by increased plant growth, at least in the early stages. It should be added that in no case which has come under our observation has a solution with a pH value greater than 7 produced yields of plant as great as those obtained from slightly acid solutions. In a paper just published, Bryan<sup>6</sup> concludes that alfalfa and clover produce maximum growth at pH 7 and pH 8. In these investigations, however, it is possible that the pH values of the alkaline solutions (quartz sand cultures were used) decreased very rapidly, especially in the zones immediately in contact with the absorbing root membranes, as a result of selective absorption and high concentration of  $\text{CO}_2$ . Our own results with alfalfa in solution culture indicate that pH 8 is less favorable to growth than a slightly acid reaction. It does not follow, of course, that plants do not exist which can grow well in an alkaline medium. In fact, we have found that Bermuda grass thrives even at pH 9. This statement would also apply, no doubt, to numerous other alkali tolerant plants.

Another question which may be raised in this connection is concerned with the necessity of providing in the soil or culture solution a supply of carbonates or bicarbonates. It has been stated, for example, that calcium must be present in the form of carbonates or bicarbonates in order to effect the required neutralization of acid developed within the plant as a by-product of the nitrogen or carbohydrate metabolism. In the culture solutions referred to in this paper, no carbonates or bicarbonates were included, and yet all the species of plants tested have made excellent growth. It is true that bicarbonates are soon formed in solutions of this type, but this is a result of the plant's own metabolism. Furthermore, certain plants, including several legumes, have been grown in solutions continuously maintained at pH values of 4.5-5.0, by the frequent addition of suitable amounts of acid without any ill effects on the plant. In such

solutions only very small amounts of bicarbonate ion are present. For these reasons, it is difficult to reach the conclusion that bicarbonates are in general essential for the growth of many common agricultural plants, at least when nitrogen is supplied in the form of nitrate. Certainly, the plants which we have experimented with can easily effect the formation of bicarbonate in such solutions. No doubt, small quantities of bicarbonate ion may be present in the juices of many plants, as suggested by Haas,<sup>7</sup> but the metabolism of the plant could account for this condition, irrespective of external sources of bicarbonate ion. When nitrogen is supplied to the plant in the form of ammonium salts, sulphate or chloride, the culture solution may reach an acidity of pH 3.2, according to certain experiments on barley plants carried on in this laboratory. This observation is in general agreement with Olsen's<sup>8</sup> recent results on other plants. The presence of a suspension of calcium carbonate prevents the solution from reaching this injurious acidity, but it is not essential to relate the improved environment to the presence of calcium bicarbonate as such, since other basic ions are also in equilibrium with bicarbonate ion, and take part in the buffer system of the plant. Calcium, of course, may possibly play a special role in some plants by virtue of its ability to form insoluble salts with certain organic acids. To what extent such precipitations occur in the common agricultural plants remains to be shown, but, in any event, it does not appear to be justifiable to assign to calcium an exclusive role in the regulation of the reaction of plant juices. Potassium, for example, in equilibrium with organic acid anions, must possess a buffer effect of very important magnitude. As a matter of fact, such plants as barley and peas may show a slightly decreased acidity in their juices when the supply of calcium in the culture solution is restricted, as evidenced by a number of experiments made in this laboratory by Newton.<sup>11</sup> Haas<sup>7</sup> also cites several similar cases based on soil studies.

In conducting experiments with alkaline culture solutions, several serious difficulties are confronted. In the first place, certain of the essential constituents, notably calcium, iron and phosphate, may precipitate out. It is also not a simple matter to maintain a given alkalinity in the solution, because of the marked tendency of the plant to decrease the OH ion concentration. It is not ordinarily feasible to use a solution of sufficiently high buffer value which



is otherwise adapted to plant growth. Possibly the best method would involve the principle of a continuously flowing solution, but such an arrangement is not always practicable. In this laboratory, Theron<sup>5</sup> carried on a series of experiments in which very large volumes of solutions were employed and alkali or acid added frequently (twice daily when necessary) so as to maintain with fair constancy the desired reaction. These data will be reported elsewhere in more detail, but it is desirable to state here that a greater proportion of cations was removed from the alkaline solutions than from those with an acid reaction. The decrease of alkalinity was the result of this selective absorption of bases and also of the excretion of carbon dioxide from the plant roots. The decrease of alkalinity in various single salt solutions is made manifest by certain of the data cited in this paper. It may be well to emphasize again the fact that when sand cultures are employed, the maintenance of a constant alkaline reaction in a solution in immediate contact with roots is almost impossible when solutions of suitable concentration are used. This fact must be taken into account in the interpretation of sand culture experiments.

The solutions so far under discussion have been complete culture solutions and are, therefore, of extremely complex nature. It is frequently interesting and enlightening to experiment with single salt solutions (compare Gericke<sup>10</sup>). In some preliminary tests made a number of years ago, the effect of the plant on the reaction of several such solutions was observed, and it was concluded that no injurious intensity of acidity or alkalinity was developed. In these experiments, the plants were grown for six or seven weeks in complete culture solutions and were then transferred to the solutions of single salts, after rinsing the roots of the plants in distilled water. In continuance of this work, plants have been transferred to the single salt solutions immediately after germination, or after only a limited period of contact with the complete culture solution. Under these circumstances more extensive changes of reaction have occurred in various salt solutions. The plants were grown in vessels of 120 c.c. capacity, from thirty to fifty plants generally being used for each solution. The cultures were carried on at various times of the year, in most instances outdoors, but in several experiments the plants were placed in a heated greenhouse. The reactions were determined on composite samples by standard colorimetric methods. The buffer

effect of the solutions were frequently very slight, and in such cases the values obtained can be regarded as only approximate. Definite changes of reaction produced by the plant can be demonstrated, however, without difficulty. The total concentrations of the solutions were of magnitudes found in many culture and soil solutions.

Three types of plants were used in the present experiments: Barley (Beldi and Mariout variety), peas (Dwarf Wonder variety), cucumbers (White Spine variety).

The measurements of the reactions of the different solutions, after various periods of contact with different plants, are given in tables 1 to 4. These data show that solutions of certain salts were consistently changed to a reaction more acid than the initial one, as a result of even relatively brief contact with the roots of the plants whether of barley, peas or cucumbers (tables 2, 3, 4). The ammonium salts are particularly subject to an increase of hydrogen ion concentration. The other solutions in which a significant decrease of pH value was generally brought about include  $K_2SO_4$ ,  $Na_2SO_4$ ,  $KH_2SO_4$ . Peas, barley, and cucumbers all exhibited this tendency. Breazeale<sup>11</sup> by titration methods, showed that an increased acidity was produced in  $K_2SO_4$  solutions by the growth of wheat seedlings. A very interesting instance of increase of acidity was furnished by cucumber plants growing in solutions of calcium salts. In a number of cases, a very definite decrease of pH value took place. This effect was also found in one experiment with peas grown in similar solutions. It does not follow, however, that peas will absorb more Ca than barley from a complete culture solution. This was not found to be the case in the experiments of Newton.<sup>9</sup>

The solutions of the following salts tended to have their hydrogen ion concentrations decreased when barley plants were grown in them:  $(Ca(NO_3)_2)$ ,  $CaCl_2$ ,  $Mg(NO_3)_2$ ,  $NaNO_3$ ,  $Ca(H_2PO_4)_2$ ,  $Mg(H_2PO_4)_2$ ,  $NaH_2PO_4$ . In the case of any nitrate (except ammonium), the solution generally attains a reaction very close to neutrality, but the precise reaction will be determined by the quantity of free  $CO_2$  present. Under favorable weather conditions the growth of peas and cucumbers caused an increase of acidity in  $CaSO_4$  solutions to pH 4.0.

It is important to note that the changes of reaction of certain salt solutions are not always the same in the different experiments. Obviously the rate of growth and of ion absorption will determine



the rate at which a given solution can undergo a modification of hydrogen ion concentration, so that the factors of light and temperature are necessarily involved. For example, it was noted in one experiment that even a very slight shading of barley cultures markedly diminished the rate of increase of acidity in a  $K_2SO_4$  solution. After a short period of growth, the cultures nearest the window had a pH value of 4.0, and those at the opposite end of the row a pH value of 5.7. It would appear in a few cases that not only the rate but the direction of the change of reaction may be altered, according to the rate of growth. In the case of sodium and potassium sulphates, of various nitrates, and of ammonium salts, the effects are, in general, consistent for the different plants and under varying conditions of growth.

When non-buffered salts are used and at reactions where carbon dioxide has an important influence, it is not justifiable to place any emphasis on small fluctuations of hydrogen ion concentration, since this will vary with the rate at which carbon dioxide is given off by the roots and also with the temperature of the solution. The solutions having the higher acidities (pH 4.0 and less) were not influenced by this factor, since the reaction was not changed by boiling.

The effect of the plant on the reaction of the culture medium is of interest to any consideration of the absorption of ions by the plant. One of the most striking facts connected with this absorption is the ease with which  $NO_3$  ions are removed from solution and replaced by  $HCO_3$  ions. One can only speculate concerning the mechanism involved. The effect would be the same whether H ions and  $NO_3$  ions were removed, leaving the cations and  $HCO_3$  ions, formed as a result of  $CO_2$  excretion by the plant, or whether there were some sort of exchange of  $HCO_3$  and  $NO_3$  ions. This replacement of an anion by  $HCO_3$  ion is not necessarily limited to the nitrate ion. When actively growing barley plants (previously in contact with a complete culture solution) were placed in a solution of  $CaCl_2$ , more chlorine than calcium was absorbed and very appreciable amounts of  $HCO_3$  ion were formed. Rudolfs<sup>12</sup> has recently investigated the effects of contact of different solutions with the seeds of different plants. In general, there was tendency for a slightly increased but definite acidity to develop in all solutions. The change of reaction in nitrate solutions was, therefore, different from that produced by growing plants.

From a solution of  $K_2SO_4$ , either K and OH ions (or  $HCO_3$  ions) are removed, or else K ion is exchanged for hydrogen ion. The solution being initially acid from the presence of carbonic acid, the concentration of OH ions must necessarily be very small, which would presumably operate to restrict the absorption of K ion, accompanied by OH ion.

Particular interest attaches to the absorption of ammonium ions. This ion seems to penetrate into the living cell with the greatest ease. Jacobs<sup>13</sup> has shown this by direct observations on certain plant and animal cells, and Davis and the writer<sup>14</sup> have likewise found that the reaction of the vacuolar sap of *Nitella* (a fresh water alga producing large cells from which practically uncontaminated sap may be obtained) can readily be changed when the cells are immersed in even very dilute solutions of ammonium salts. The consequence is that the cell is quickly injured. As already stated, an increase of acidity was readily brought about in solutions of ammonium salts by the growth of barley, peas or cucumbers.

If one ion is absorbed more rapidly than the accompanying oppositely charged ion, it might be thought that the reaction of the root juices would also undergo change as well as that of the solution. In some of the experiments, determinations were made of the approximate hydrogen ion concentrations of the juices expressed from roots grown in solutions of various salts. There were some marked variations, but, in general, it was not possible directly to relate the change of reaction to the effect of the ion absorbed in excess. For example, the absorption of ammonium ion did not increase the alkalinity of the root juices, as far as could be ascertained. Probably this increase should not be expected in view of the influence of the buffer system of the plant, the immediate translocation of substances away from the root and the rapid metabolism of nitrogen compounds. After the elapse of sufficient time, however, the acidity induced in certain solutions ( $KH_2SO_4$ ,  $(NH_4)_2SO_4$ , and others), caused the reaction of the root juices to become more acid. In such cases, the roots were always definitely injured. With peas, it was noted that injury and discoloration affected the roots of the plants grown in solutions of  $KNO_3$  and  $NaNO_3$ , but not of  $Ca(NO_3)_2$ . The juices from these injured roots exhibited a slightly higher alkalinity than normal. The hypothesis may be advanced that an alkaline residue was formed as a result of the rapid reduction or translocation of  $NO_3$  ion.

It may be of interest to add several additional comments on the effects of various salts on root development. With barley, better development was obtained in a potassium nitrate solution than in a calcium nitrate solution. All calcium salts permitted excellent development of the roots of peas and cucumbers. In both cases, solutions containing calcium carbonate in suspension offered a very favorable medium for root growth, but it was not appreciably superior to a solution of calcium sulphate. It should be stated that the solutions containing an excess of calcium carbonate may, nevertheless, have a slightly acid reaction, because of the carbon dioxide given off by the plant roots. Cucumbers were extremely sensitive to sodium salts, so much so that any determinations of changes of reaction in such solutions are not of much value. With all three plants, injury was pronounced in those solutions which attained an acidity represented by a pH value of less than 4.

#### DISCUSSION

The data presented in this paper justify the conclusions previously reached with regard to complete or partial culture solutions containing nitrate, namely that there is a very general tendency for the plant to change the reaction toward the neutral point, whether the initial reaction be acid or alkaline. The results obtained with certain single salts make it necessary to modify the previous conclusion that the plant does not cause a solution to change its reaction to a point injuriously acid. This did not occur, in fact, in earlier experiments, with plants grown for some time in complete culture solutions and which continued to grow in the single salt solutions without injury, but when seedlings are transferred directly to the solutions (or after only a limited contact with the complete culture solution) of such salts as  $(\text{NH}_4)_2\text{SO}_4$  or  $\text{K}_2\text{SO}_4$ , certainly the acidity developed was detrimental to root growth, and eventually highly injurious to the plant as a whole.

The actual excess absorption of one ion is necessarily very slight. Acidities greater than pH 3.0 or 3.2 were not observed to develop, and this concentration of hydrogen ion would correspond to only an extremely small selective removal (or exchange) of cations. It is true that frequently a very substantial excess of cation is actually removed from the solution as shown by chemical analysis, but it has

been shown that in these solutions much of the discrepancy can be accounted for by the loss from the plant of some other cation. In other words, a sort of exchange of bases takes place. (Whether or not this occurs in the cell wall remains to be demonstrated.) Furthermore, a slowly diffusing anion, especially sulphate ion, retards very materially the absorption of the associated cation. In this connection, attention is directed to the fact that these properties of the ions are not to be explained on the simple assumption that certain ions are required by the plant and are, therefore, "selected" out of the solution. The increase of acidity may be produced in sodium sulphate, as well as in potassium sulphate solutions. Chlorine ion is removed from solution, by some plants at least, much more readily than sulphate ion. Many experiments have been carried out bearing on these statements but the results can be presented more conveniently in a later discussion.

The absorption of ions by a plant cell is by no means a matter of simple chemical equilibrium. The general nature of the problems can be most clearly illustrated by a reference to some recent experiments on *Nitella*.<sup>14 15 16 17</sup> This plant produces cells sufficiently large so that sap may be obtained from individual cells practically uncontaminated. The investigations with *Nitella* prove conclusively that the major portions of the inorganic elements contained in this plant exist as dissociated compounds. Ions are absorbed into the cell sap from an outside solution of much lower concentration. In other words, the plant must apparently do work in connection with this absorption. Possibly, however, the first step in the process is the formation of some sort of an ion compound, and this may be dependent, at least in the case of some ions, on the hydrogen ion concentration of the solution. It was found that nitrate penetrated far more rapidly into the cell sap of *Nitella* from an acid solution than from an alkaline solution. These remarks are included in this discussion because it is desired to emphasize the dynamic nature of the processes of ion absorption and of the change of reaction induced in solutions by the plant. It is also of interest to suggest the importance of Loeb's work on the chemical nature of proteins in this general connection. The maintenance of a suitable reaction within the cell is probably of paramount importance, because the hydrogen ion concentration may be one of the chief variables governing the colloidal behavior of the protoplasm. In addition to this, if any intermediate



combinations of protein and ions should be concerned in the mechanism of absorption, these must be intimately related to the hydrogen ion concentration of the solution.

Briefly referring to the soil phase of the question, it is doubtful whether the activities of the plant in the absorption of ions ordinarily bring about directly an increase of acidity in the soil. The principal source of nitrogen is generally in the form of nitrate and this ion will normally be absorbed with such rapidity that bicarbonate residues are left. Observations on soil extracts and displaced solutions give evidence that during normal crop growth and at the time of maximum absorption, practically every trace of nitrate is removed from the soil solution. The cations are also reduced in concentration to a greater or less degree but certainly not to so great an extent as the nitrate ion. Therefore, it would seem necessary to conclude that bicarbonate ion is correspondingly increased in concentration, just as it is in solution cultures. The continued use of ammonium salts on a soil generally causes, in course of time, an increased hydrogen ion concentration, but here it is essential to consider also the activities of micro-organisms, which is not within the scope of this paper.

#### SUMMARY

1. The reaction of a culture solution has an important bearing on the absorption of ions by plants. The absorption of  $\text{NO}_3$  ion was found to be favored by an acid reaction, and a relatively increased absorption of cations occurred when an alkaline reaction was present.

2. Observations were made of the effect produced on the reaction of various single salt solution by the growth of barley, peas, and cucumbers. An increase of acidity occurred with a number of salts, particularly ammonium sulphate and chloride, potassium or sodium sulphates, and potassium phosphate. Injury to the plant was produced by the acidity developed.

3. The importance of light and temperature as influencing the absorption of ions and change of reaction in culture solutions, is emphasized.

4. The necessity for considering the part taken by  $\text{CO}_2$  and by  $\text{HCO}_3$  ion in any explanation of the effect of the plant on the reaction of culture solutions is pointed out.

5.  $\text{KNO}_3$  in single salt solution caused injury to the roots of pea plants, but not to those of barley.

6. The general question of the relation between ion absorption and hydrogen ion concentration is briefly discussed. Attention is called to the possible bearing of Loeb's work on proteins in this connection.

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TABLE 1  
ABSORPTIONS OF IONS BY BARLEY PLANTS FROM ACID AND NEUTRAL SOLUTIONS  
(Computed in milli-equivalents, absorbed by each culture during periods of one week)

Period of growth	Initial pH value	K	Ca	Mg	NO <sub>3</sub>	PO <sub>4</sub>	SO <sub>4</sub>	Total positive ions	Total negative ions	Differ- ence*	Sum of positive and negative ions
4-5 weeks.....	5.1	2.30	2.40	.66	6.18	.63	.87	5.36	7.68	-2.32	13.04
	6.8	1.66	1.40	.74	2.53	.45	.48	3.80	3.46	+ .34	7.26
6-7 weeks.....	5.1	3.23	4.59	1.64	8.62	1.13	1.48	9.46	11.23	-1.77	20.69
	6.8	3.20	3.04	1.32	6.70	.64	1.35	7.56	8.69	-1.13	16.25
11-12 weeks.....	5.1	2.74	4.19	1.40	8.73	1.53	1.50	8.33	11.81	-3.48	20.14
	6.8	2.87	3.69	1.89	5.23	.98	1.68	8.45	7.89	+ .56	16.34
Composition of Original Solution.....	5.1	6.35	14.80	7.40	17.70	1.89	7.16	(Small quantities bicarbonate and silicate ions also present)			
	6.8	6.45	14.30	8.38	16.10	1.39	8.22				

20 cultures and 40 plants used for each solution

\* Referable to undetermined bicarbonate ion chiefly, not to errors in analysis.



TABLE 3  
EFFECT OF PLANT ON REACTION OF SOLUTION

Salt	Concen- tration, Milli- equiv.	pH Initial	EXPERIMENT 1* (Peas)					EXPERIMENT 2 (Cucumbers)			EXPERIMENT 3 (Barley)	
			pH Values					pH Values			pH Values	
			After 3 days	After 6 days	After 13 days	After 17 days	After 21 days	After 5 days	After 9 days	After 13 days	After 7 days	After 11 days
KNO <sub>3</sub> .....	10.0	5.0	5.4	5.0	5.4	6.2	6.4	5.6	5.0	5.2	5.4	6.6
KCL.....	10.0	5.2	4.8	4.8	4.8	5.0	6.0	5.2	4.2	5.0	4.8	4.0
K <sub>2</sub> SO <sub>4</sub> .....	10.0	5.2	5.0	4.8	4.4	4.4	4.4	5.0	4.4	5.0	3.8	3.4
KH <sub>2</sub> PO <sub>4</sub> .....	10.0	4.6	4.8	4.6	4.4	4.2	4.2	4.6	4.6	4.6	3.6	3.6
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	10.0	5.0	5.6	5.8	5.8	6.8	6.6	5.8	5.6	4.6	6.8	6.8
CaCl <sub>2</sub> .....	10.0	5.2	5.0	5.0	5.0	5.2	5.6	5.2	5.2	5.0	5.2	5.4
CaSO <sub>4</sub> .....	10.0	6.0	5.2	6.0	5.4	5.8	5.2	5.2	4.6	4.0	5.6	4.8
NaNO <sub>3</sub> .....	10.0	4.6	5.4	5.2	5.4	5.8	6.0	5.8	5.8	6.4	5.6	6.2
NaCl.....	10.0	5.0	5.0	4.8	5.0	5.4	5.0	5.8	5.6	5.8	4.2	4.0
Na <sub>2</sub> SO <sub>4</sub> .....	10.0	5.2	5.0	5.2	5.0	5.6	6.0	5.8	5.0	5.2	3.8	3.6
NH <sub>4</sub> NO <sub>3</sub> .....	10.0	5.0	5.2	5.0	5.0	5.2	5.4	5.2	5.0	4.4	4.0	5.0
NH <sub>4</sub> Cl.....	10.0	5.2	4.8	4.6	5.0	5.0	5.0	5.0	4.8	4.0	3.4	3.2
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	10.0	5.0	5.2	4.8	5.0	5.0	.....	5.0	4.8	4.0	3.4	3.2

Seedlings placed in single salt solutions immediately after germination

\*Unfavorable weather conditions

TABLE 4  
EFFECT OF PLANT ON REACTION OF SOLUTION

EXPERIMENT 1—(Peas)					EXPERIMENT 2—(Peas)						
Salt	Concentration, Milli-equiv.	pH Values				Salt	Concentration, Milli-equiv.	pH Values			
		Initial	After 4 days	After 12 days	After 19 days			Initial	After 7 days	After 15 days	After 23 days
NH <sub>4</sub> NO <sub>3</sub> .....	10.0	5.8	4.4	4.3	4.2	KNO <sub>3</sub> .....	10.0	5.8	5.2	5.6	6.6
NH <sub>4</sub> Cl.....	10.0	5.8	4.0	3.9	4.2	K <sub>2</sub> SO <sub>4</sub> .....	10.0	5.8	4.6	4.0	3.9
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	10.0	5.8	4.2	4.2	4.3	N <sub>2</sub> SO <sub>4</sub> .....	10.0	6.0	4.6	4.4	4.3
						CaCl <sub>2</sub> .....	10.0	5.8	5.2	5.0	4.2
						CaSO <sub>4</sub> .....	10.0	5.8	5.4	5.1	3.9
						NH <sub>4</sub> NO <sub>3</sub> .....	10.0	5.8	4.8	4.9	5.1
						NH <sub>4</sub> Cl.....	10.0	5.8	4.3	4.1	4.2
						(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	10.0	5.8	4.3	4.1	4.1

Seedlings placed in single salt solutions immediately after germination

Seedlings placed in single salt solutions immediately after germination

EXPERIMENT 3—(Cucumbers)					EXPERIMENT 4—(Cucumbers)					
Salt	Concentration, Milli-equiv.	pH Values				Salt	Concentration, Milli-equiv.	pH Values		
		Initial	After 9 days	After 17 days	After 21 days			Initial	After 6 days	After 14 days
KNO <sub>3</sub> .....	10.0	5.1	5.5	5.8	5.6	KNO <sub>3</sub> .....	10.0	5.2	6.1	.....
KCl.....	10.0	4.9	4.5	4.3	4.1	K <sub>2</sub> SO <sub>4</sub> .....	10.0	5.2	4.2	3.9
K <sub>2</sub> SO <sub>4</sub> .....	10.0	5.0	4.2	4.3	4.3	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	10.0	5.4	6.5	6.8
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	10.0	5.0	5.8	5.6	5.4	CaCl <sub>2</sub> .....	10.0	5.4	5.8	6.0
CaCl <sub>2</sub> .....	10.0	5.0	5.5	5.0	4.2	CaSO <sub>4</sub> .....	10.0	5.4	5.2	4.2
CaSO <sub>4</sub> .....	10.0	5.6	5.6	5.0	4.2	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	10.0	5.2	5.1	5.1

Seedlings placed in single salt solutions immediately after germination

Unfavorable growing conditions  
Seedlings placed in single salt solutions immediately after germination

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TECHNICAL PAPER No. 13

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SOME MUTUAL EFFECTS ON SOIL  
AND PLANT INDUCED BY  
ADDED SOLUTES

BY

JOHN S. BURD AND J. C. MARTIN

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A recent paper<sup>5</sup> dealing with the effects of salts on soils states that "the solid, liquid and gaseous phases of a soil form a chemical system and a material change in any one of these phases must inevitably affect the equilibrium of the system." Application to the soil of fertilizers, irrigation waters carrying soluble salts, or of any substance capable of dissolving in water will obviously affect the liquid phase and hence modify the soil's equilibrium. A most important consequence of the recognition of the fact that the soil comprises a dynamic system is that the investigator may no longer hope to correlate plant production directly with the amounts of the specific chemical elements or of salts applied to the soil. Such correlations as are developed must be between production and increases or decreases in the amounts of the various solutes in the effective solution of the soil.

Working with 0.01 N. solutions in the proportion of five parts of solution to one of soil, Kelley and Cummins<sup>5</sup> have shown that, after one hour of contact:

"1. Chemically equivalent solutions of the chlorides, sulfates and nitrates of a given base, produced substantially equivalent chemical reactions in the soils studied.

"2. The solubility of the anion of the neutral solutions was not materially affected by the soils studied, but an exchange of bases took place, with the result that a portion of the base of the added salt passed out of solution and a chemically equivalent amount of other bases was set free from the soil silicates."

In the experiments upon which these conclusions were based, there was no change in reaction in any of the soils, and, as stated, the anions were not materially affected. The evidence is fairly convincing that, under such circumstances, the interaction of salts with soils is in general a strictly stoichiometric one.

In this laboratory, working with several soils (the same soils discussed hereinafter with reference to other experiments), one of us has shown that when the soils contained large quantities of solutes and were mixed with small amounts of potassium chlorid, the potassium fixed was equivalent to the amounts of calcium, magnesium, and sodium brought into solution. As the proportion of potassium chlorid to soil was increased, the agreement between the reaction values of potassium removed and those of other bases entering the solution was fair, but not in all cases conclusive of an exact equivalence. Using the same soils after very thorough leaching and with the same proportions of potassium chlorid to soil as in the former experiment, the agreement between the reaction value of potassium fixed and other bases rendered soluble was good in the case of only one soil, and then only when the smallest proportion of added potassium chlorid was involved. In these experiments, no measurements of H ion or anion concentration were made so that a satisfactory analysis of the causes of the discrepancy is not possible. In the light of the conclusions quoted above, however, it seems probable that this inconsistency in our own results is more apparent than real and that the interchanges between added salts or solutions and soils in general are strictly stoichiometric, if all of the ions involved, including H or OH ion, are taken into account. To state that such interchanges take place in equivalent proportions and depend upon familiar chemical principles is satisfactory so far as it goes, but, because of the complexity of the systems involved, leaves a great deal to be learned as to the specific effects of salt or fertilizer treatments upon individual soils. The data referred to herein, as well as much other evidence that might be adduced, indicate a definite tendency toward an increase of water soluble constituents when salts are added to soils. This increase is obviously due, in part, to the added constituents which are not rendered insoluble by reaction with liquid or solid phase soil constituents and in part to new soluble material originating in the solid phase of the soil itself. Little is known as to the degree of permanence of the

new equilibria engendered by salt or fertilizer applications, and there is good reason to believe that these may be more or less transitory, particularly when the additions are small in magnitude. If this be true, the bearing of the factor of time upon ionic interchanges between soil and solution must be more completely studied if conclusions are to be drawn as to the ultimate effects of salt or fertilizer treatments on plant production. Moreover, the liquid phase of such treated soils must be studied concurrently with the growth of crops. The object of the present study was, therefore, to ascertain: first, the general qualitative and quantitative effects of salt and fertilizer treatments on the water extractable constituents of soils after the lapse of a considerable period of time (in this case eight months); and second, the effect of changes induced by salt and fertilizer treatments upon plant withdrawals and production. It is evident that comprehensive conclusions from this type of experimentation can be obtained only from a very large number of systematic studies of the effects of single salts on each of a great many soils. Such a method of attack is obviously beyond the facilities of any ordinary laboratory within a reasonable period of time, and we have deemed it necessary in the present state of our knowledge to use a small number of more or less complex treatments on a few soils. While such a procedure necessarily limits the conclusions, it may be expected to yield more facts and at the same time supply data for the proper formulation of systematic experiments of more restricted scope.

#### PRELIMINARY EXPERIMENTS

These consisted of a study of the effects of certain treatments on the water soluble constituents of two soils, one a silty clay loam known as No. 1c and one a sandy loam known as No. 15. The soil, after admixture, was kept for eight months in loosely covered jars permitting air circulation, the moisture content of the soils being maintained at 22 per cent and 15 per cent, respectively. The treatments included additions to each soil of (1) varied concentrations of a complete culture solution commonly used in this laboratory; (2) varied amounts of potassium nitrate and acid phosphate (superphosphate); and (3) certain amounts of tankage. The last named material, since it contained no water soluble constituents, was not expected to shed

light on chemical interrelations but was included because of the opportunity offered to compare its effects with those of the added salts.

No attempt was made to correlate the amounts of the various solutes added in the culture solution with those of field practice. It should be noted, however, that the amounts of constituents added in the minimum treatment with solutions (22 p.p.m. K, 77 p.p.m.  $\text{NO}_3$ , and 10 p.p.m.  $\text{PO}_4$ , corresponding approximately to 88, 308, and 40 pounds per acre foot), although fairly heavy for nitrate, would not constitute prohibitive applications in intensive fertilizer practice. Attention is called to this, not for the purpose of attempting to justify correlating the results of this experiment with field practice, but to indicate that the amounts of salts added in the minimum treatments were not so excessive as to constitute a highly artificial dosage. In this connection, we may also state that the minimum potassium nitrate-superphosphate and tankage treatments, while probably exceeding the economic limit under most circumstances, are not greatly in excess thereof.

The data obtained are presented in table 1.

#### EFFECT OF ADDED SALTS ON WATER EXTRACTABLE SOLUTES AFTER EIGHT MONTHS

In dealing with the results obtained by adding a mixture of salts to the soil complex, it is evident that one may not hope to completely differentiate between the effects of a given element or the single salt from which it is derived and those induced by reactions between the other added salts and the soil minerals. The effect of common ions in reaction has, however, such importance in determining solubilities as to require that the data be studied, in the first instance at least, in the light of the relation of specific elements in the added solutions to changes in the condition of the same elements in the soil.

We have attempted to classify the results obtained upon the basis of types of behavior in the relation between the amounts of solutes added and those extracted by water from the treated soils. Four distinct types of behavior may be noted:

1. The water extractable solute is increased in the treated soil, but the amount of the increase is not equal to the amount of the given solute in the added solution or salt.



Such behavior is shown by potassium and calcium in practically all instances in both soils; by nitrate in all instances in both soils with the exception of two treatments in soil 1c; and by magnesium and sulfate in soil 15. In all of these instances, except in the case of nitrate, the discrepancy between the amount of added solute and its increase in the water extract may be accounted for by removal of a portion of the solute by solid phase material or by actual precipitation. Whatever the cause of these losses of potentially soluble material in the case of any given soil, the result is fixation of a considerable proportion of the added constituent in a form which is not readily soluble in water. The ultimate effect on plant growth of the material so fixed can only be surmised. The important and significant effect to be noted here is the ease with which the content of materials readily soluble in water may be enhanced in the soil, not only temporarily, but for a period of many months. The losses of soluble nitrogen ( $\text{NO}_3$ ) cannot be accounted for in the manner indicated above, but must be ascribed to the influence of biological factors. The result, however, is consistent with much that is known and points to continuous and substantial losses of nitrate nitrogen in soils in general.

2. The water extractable solute is increased in the treated soils by an amount that is greater than that added in the form of a solution or soluble salts. A special case is that in which the solute in question is entirely lacking in the added solution or salts.

This behavior is shown by sulfate and magnesium in one of the soils (1c). Such an effect may be reasonably assigned to ionic interchanges between added solutes and solid phase material in the soil complex or to increased solubility of solid phase material in a new soil solution. There seems to be no reason to regard it as specific to the ions named, as such an effect might have been masked in the case of other ions if added solutions or salts contained large amounts of solute, more of which was fixed than was released from the solid phase by ionic interchange. From the differences in behavior of the two soils with approximately equal concentrations of a given solute in the solution added (41 p.p.m. Mg in soil 1c and 36 p.p.m. Mg. in soil 15—see also differences in sulfates in the two soils) as well as upon theoretical grounds, it is evident that the character of the solid phase material is largely determinative of whether the increase in amount of water extractable material is greater or less than that added in soluble form.

3. The water extractable solute is decreased by the treatment.

This applies to sodium in one instance in soil 1c and in numerous instances in soil 15. Such an effect upon a constituent capable of forming highly soluble salts demands further investigation, particularly in view of the inherent error in determining small quantities of this element. Literally interpreted, however, the results suggest and indeed can only mean that prolonged contact of soil and salt solutions may result in the formation of mineralogical species from which the sodium is less readily dissolved by water than from those present in the untreated soil.

4. The water extractable solute is increased by the treatments, but the increase constitutes only a small fraction of the added solute.

This appears to be the characteristic of phosphate behavior. With small applications, the increase is negligible or doubtful, but the larger applications induce appreciable increases in water soluble phosphate. More recent investigations<sup>3</sup> in this laboratory indicate that the soluble phosphate concentrations of natural soils fluctuate from time to time. This fluctuation may be ascribed to changes in reaction, or to changes in the concentrations of cations which tend to form insoluble phosphates. Obviously, the addition of soluble phosphates should tend to upset the phosphate equilibrium and it is not surprising that an increase of water extractable phosphate should ensue in spite of the low solubility of phosphates in general.

The addition of materials initially insoluble in water cannot be expected to furnish evidence as to the nature of ionic interchanges. Indeed, the results with tankage applications indicate that such materials have very little influence on the water solubility of soil constituents other than those contained in the added material itself. In the light of common experience in fertilizer practice, it is, however, not surprising that the nitrate and water soluble phosphate of soils should be increased by tankage applications.

#### VEGETATION EXPERIMENTS

The results of the experiments reported above demonstrated to our satisfaction that the potentially soluble constituents of soils may be readily increased for a considerable period of time and this by salt or fertilizer applications which are not necessarily unusual or excessive. They also gave valuable information as to the magnitude of the



increases in soluble matter to be expected from given applications, and thus served as a general guide to the treatment of these soils in studies of the relation of such increases to plant absorption and production. One of the soils used in the preceding experiment was included in the subsequent experiments where plants were grown on treated soils. This soil (No. 15) was at the time of the experiment in a high state of fertility and it was deemed desirable to include a soil of lower fertility. Soil 1c did not conform to this requirement and another soil known as No. 21 (Oakley blow sand) was substituted in its stead. On this account, the dosage used in the various treatments of this soil was not predicated on previous experimental work. It was known, however, that the soil does not yield good crops without substantial amounts of fertilizers.

*Procedure.*—Each soil was thoroughly screened and mixed, after which weighed portions received the various treatments in the form of solutions.

Each of the treated portions was again thoroughly mixed and placed in galvanized iron tanks 60 inches by 30 inches by 18 inches deep, according to the usual practice of this laboratory.<sup>6</sup> The crop, Beldi barley, was planted immediately after the soil was installed. The tanks were placed parallel to each other, with uniform exposure to light. All the soils were watered with distilled water and maintained at optimum moisture, each season, until the crop was nearly ripe. Only one treatment was made, but two successive crops were grown. The seasons covered were 1920 and 1921 for soil No. 15, and 1921 and 1922 for soil No. 21. Water extractions of both soils under all treatments were made periodically for nearly two years, but the periods were less frequent the second year.

*Soil Treatments.*—From the results of our preliminary experiment, it is evident that while all soluble salts are likely to have important effects on the solubility of soil constituents, increases of specific solutes can be most readily and certainly brought about and maintained if the particular solute which it is desired to increase is added to the soil. On this account, in vegetation experiments of limited scope, it is desirable to treat the soil with salts capable of supplying those ions which are not only readily absorbed by the plant, but which are required in substantial quantity for its development. Such ions obviously include nitrate, phosphate, and potassium, and these were used in the form of

sodium nitrate, sodium dihydrogen phosphate, and potassium chlorid. In the treatment of soil No. 15, the attempt was made to use such amounts of the various salts as would about double the amounts extractable by water from the untreated soil. Since the amount of fixation of phosphate and potassium varies with the time of contact of solution and soil, and since losses of nitrate through biological activities are to be expected, it is obvious that attempts to double the water extractable constituent could at best only result in an approximation thereto and this for a limited period. The results, hereafter, show that we have been reasonably successful in approximately doubling the water extractable nitrate and potassium in the early stages of the experiment, but that owing to an over-estimate of the fixing power of the soil for phosphate, the addition of phosphate was, perhaps, unnecessarily large. When, as a result of the work of the first season, an additional experiment with a soil of low fertility was decided upon, it appeared necessary from what was known of the soil selected (No. 21), to add very much larger quantities of nitrate in its treatment if an adequate response in crop production was to be expected. The amount of the potassium salt treatment of this soil was left as before and the phosphate treatment diminished as suggested by the results of the work of the preceding year with soil No. 15.

The schedules of treatment are indicated in the following tables:

TABLE 2A

## SCHEDULE OF TREATMENTS AND YIELDS OF SOIL No. 15

Tank No.	Designation	Treatments in terms of:		Air-dry weight of crops (Grams per tank, 12½ square feet)	
		Pounds per acre foot	P. P. M. of water-free soil	1920	1921*
1	Half nitrate.....	111 NaNO <sub>3</sub>	20 NO <sub>3</sub>	830	Discontinued
2	Nitrate.....	221 NaNO <sub>3</sub>	40 NO <sub>3</sub>	895	475
3	Nitrate-Phosphate.....	221 NaNO <sub>3</sub>	40 NO <sub>3</sub>		
		1010 NaH <sub>2</sub> PO <sub>4</sub>	198 PO <sub>4</sub>	1071	552
4	Untreated.....			811	425
5	Phosphate.....	1010 NaH <sub>2</sub> PO <sub>4</sub>	198 PO <sub>4</sub>	908	436
6	Half phosphate.....	505 NaH <sub>2</sub> PO <sub>4</sub>	99 PO <sub>4</sub>	921	Discontinued
7	Potassium.....	768 KC1	100 K	816	Discontinued
8	Half potassium.....	384 KC1	50 K	844	Discontinued

\* The crop was attacked by a fungus disease and the low yields in this column are not regarded as conclusive of a falling off in fertility (see results from Soil No. 21 for such an effect).

TABLE 2B

SCHEDULE OF TREATMENTS AND YIELDS OF SOIL NO. 21

Tank No.	Designation	Treatments in terms of:		Air-dry weight of crops (Grams per tank, 12½ square feet)	
		Pounds per acre foot	P. P. M. of water-free soil	1921	1922
1	Nitrate.....	554 NaNO <sub>3</sub>	100 NO <sub>3</sub>	1144	418
2	Nitrate-phosphate.....	554 NaNO <sub>3</sub> 630 NaH <sub>2</sub> PO <sub>4</sub>	100 NO <sub>3</sub> 124 PO <sub>4</sub>	1272	407
3	Untreated.....	.....	.....	368	261
4	Phosphate.....	630 NaH <sub>2</sub> PO <sub>4</sub> ....	124 PO <sub>4</sub>	799	134
5	Potassium.....	768 KCL	100 K	820	306
6	Untreated, uncropped	.....	.....	.....	.....

DISCUSSION OF RESULTS

The discussion of results is conveniently divided into two parts: first, a consideration of the effects of the added salts on the condition of the various elements in the soil from time to time as indicated by the results of water extraction; second, such correlations as may appear between the amounts of the various solutes actually absorbed by the crop and the amounts found by water extraction of the soil on the one hand, or by the amounts of dry matter production on the other. This division permits of the separate graphic representation (figs. 1 to 5) of the effects of salt additions on water extractable soil constituents and of a brief tabular presentation of the results of absorption by the crop (tables 3A and 3B).

In considering the graphic representation of effects on the soil, it should be understood that the results from the minimum applications (half nitrate, half phosphate and half potassium) are omitted from the graphs in order to avoid confusion in following the lines representing different treatments. Since these data are not included elsewhere, it is necessary to explain that these smaller treatments generally produced qualitative effects similar to those brought about by the larger applications, but that these effects were uniformly of smaller magnitude.

In further explanation of the graphs, attention should be drawn to the fact that the work with soil No. 15 did not include an untreated uncropped, or fallow check. To those who are unfamiliar with the

previous work from this laboratory<sup>6</sup> covering the behavior of water extractable solutes in cropped and uncropped soils without fertilizers, it should be stated that uncropped soils invariably maintain themselves at higher levels than do their cropped duplicates.

In the work with soil No. 21, we have included an untreated, uncropped plot which shows the typical behavior of fallowed soils. Attention should also be called to the fact that in the season of 1922, the second year of the experiments with soil No. 21, the number of observations made were extremely limited, so that changes in direction of the lines of the graphs are doubtless more abrupt than a larger number of determinations would have shown.

*Nitrate in soils.*—An important characteristic of nitrate behavior in soils and one which has received much attention in this laboratory is that this constituent is invariably reduced to a low level and practically disappears from cropped soils at the height of the growing season (plants being 8–9 weeks old). Such an effect is to be observed in the present data in both soils for both seasons in the lines representing nitrate in the cropped, but otherwise untreated soils. While the evidence is overwhelming that nitrates tend to be reduced to a very low level in soils under crop, it is by no means certain that soils carrying very large amounts of nitrates at the beginning of the growing season or so constituted biologically as to resist losses of nitrate nitrogen, may not be able to retain substantial concentrations of nitrates throughout the growing season. The present data show, however, that considerable increases of the initial nitrate concentrations do not prevent the practical disappearance of nitrate in soils under crop. Such enhanced concentrations persist for a comparatively short time, and diminish to negligible amounts at the height of the growing season. After the harvesting of the crop, the nitrate content of the soil again increases and, independently of the previous treatment, reaches a maximum in the spring of the following year. Then, in the absence of further treatments, the nitrate behavior appears to be identical in all plots of both soils. None of the treatments appear to have substantially changed the nitrate concentrations at any time with the exception of those carrying additional nitrate and these only affect the soil for a very limited period.



*Phosphate in soils.*—The fact that the soil solution of most natural soils is probably saturated with reference to phosphate in the particular system and that the phosphate concentration of such solutions is independent of the amount of moisture present, now seems quite certain.<sup>2</sup> This does not mean that the concentrations of phosphate in a given soil may not vary from time to time. Indeed, the converse is to be expected if the reaction of the soil is modified by any cause or if there occur substantial changes in the concentration of certain cations in the liquid phase. Changes in concentration of cations capable of forming comparatively insoluble phosphates (Ca, Mg) may be brought about in cropped soils by plant absorption, by leaching, and by addition of salts tending to change the previous equilibrium. It is evident, however, that fluctuations in phosphate concentration, if small in magnitude, may not be measured with certainty by the criterion (water extraction) used in the present work. It is not surprising, therefore, that significant variations of water extractable phosphate do not appear in either of our soils, whatever the treatment, in the absence of applications of large amounts of soluble phosphate. Where such additions were made, however, the water extractable phosphate has been greatly increased over a period of two years, although it is gradually but certainly diminishing. We interpret this to mean that the equilibrium of the system, soil-soil moisture, has been modified by these large applications of sodium-dihydrogen-phosphate, but that unless the nature of the solid phase material, and particularly of the undissolved phosphates therein, has been substantially modified by the treatment, the phosphate concentrations in the soil solution will ultimately return to their former magnitudes, whatever these may have been.

*Potassium in soils.*—Addition of potassium chlorid equivalent to 100 p.p.m. of soil approximately doubled the initial water extractable potassium in both soils, which, with fluctuations, decreased somewhat at the end of the first year. The results of other treatments do not appear to have materially affected the water extractable potassium in soil No. 15. In soil No. 21 the treatments carrying phosphate diminished the water extractable potassium from the first, and this effect persisted for the entire subsequent period (nearly two years) of observation. Water extractable potassium was also decreased in the nitrate treated soil after the first few weeks, when the plants had

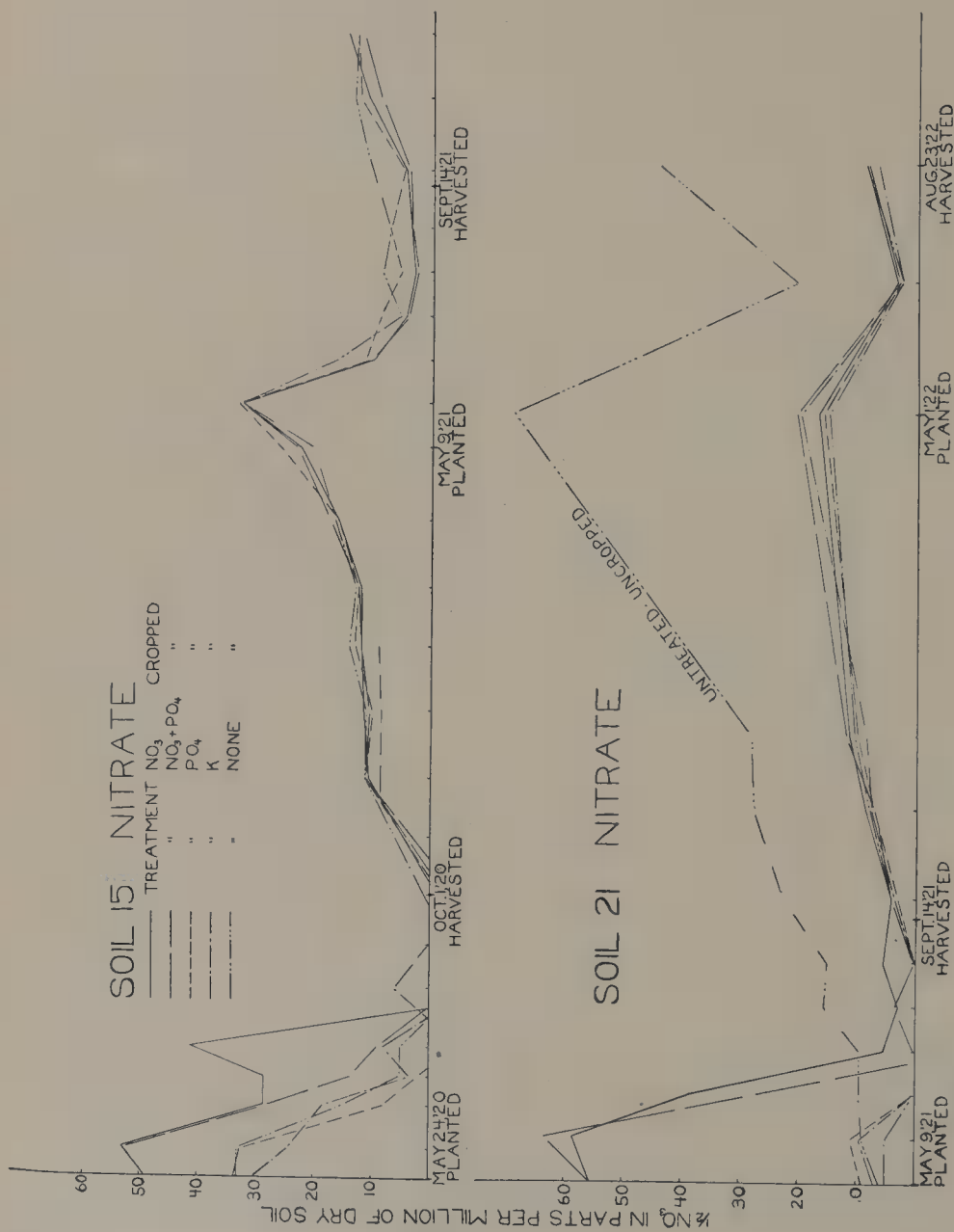


Fig. 1



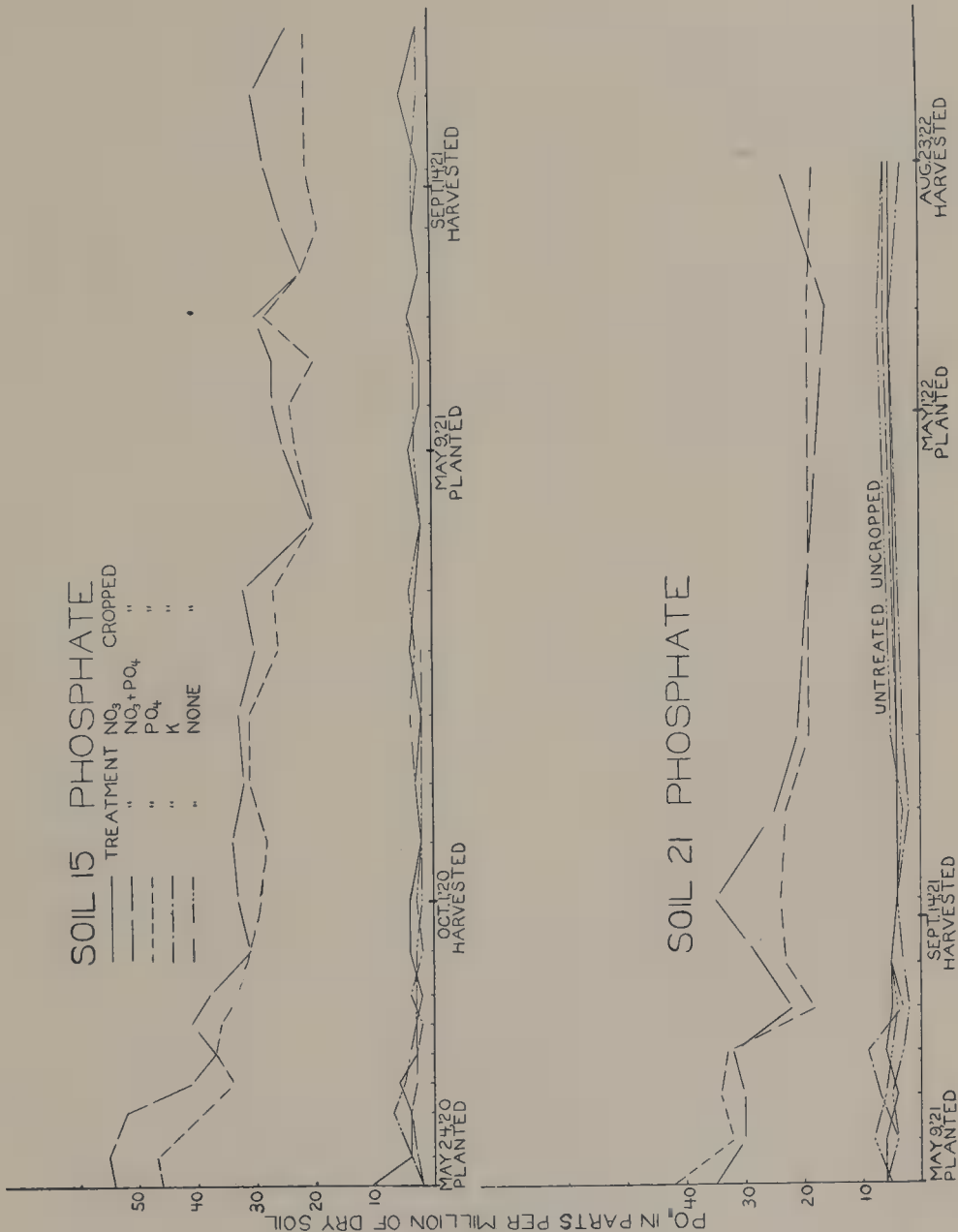


Fig. 2

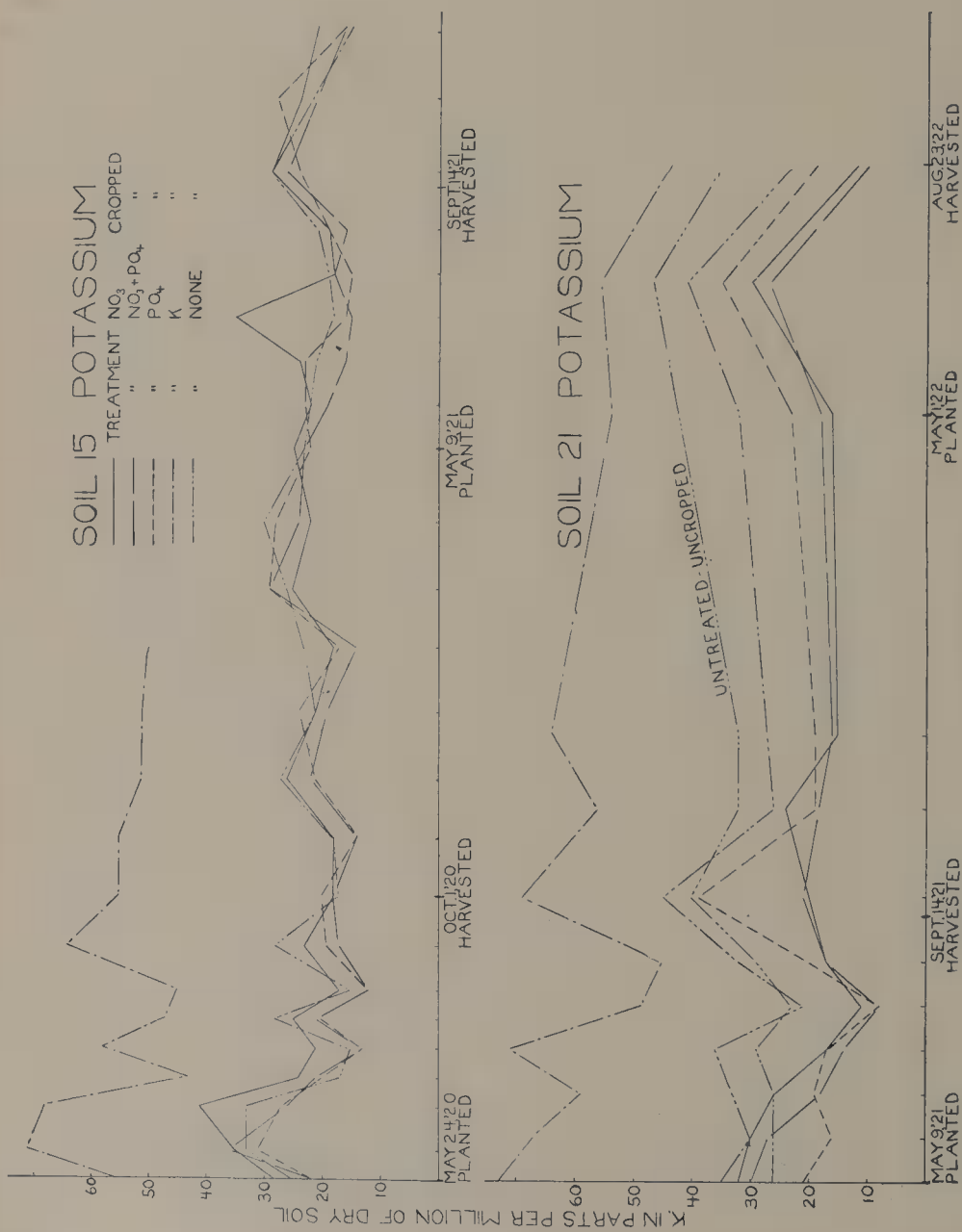


Fig. 3

reached a considerable degree of development and were doubtless actively drawing upon the potassium of the soil.<sup>1</sup> With soil No. 21 the actual amounts of potassium withdrawn from the nitrate treated and the nitrate-phosphate treated portions (table 3B) were considerably greater than those from the untreated portion and suggest that the diminished amounts of water extractable potassium found in these soils after the crop was well grown might be directly related to the growth of the crop. This would not, however, account for the diminished amount of water extractable potassium found in both of the phosphate treated portions of soil No. 21 in the first few weeks after treatment, nor to the persistently low figures obtained subsequently from the portion of soil where phosphate alone was used in the treatment and where the withdrawal of potassium by the plants was not very much larger than that from the untreated portion of soil. We can only suggest that in spite of the comparatively high solubility of potassium phosphate, there may be a partial precipitation of potassium when phosphate is added to certain equilibrium systems. This might reasonably be expected to be reflected in diminished amounts of water extractable potassium.

*Calcium and magnesium in soils.*—Since neither calcium nor magnesium salts were added to the soils, it is evident that observed changes in water extractable calcium and magnesium were caused either by plant withdrawals of these elements or by reactions between added salts and soil constituents, or by changed solubility of solid phase material in the new soil solution. Where potassium salts were added to the soils, there was a substantial increase in water extractable calcium in both soils and of water extractable magnesium in soil No. 15. This increased solubility began before the effect of plant withdrawals had had an opportunity to operate and is definitely referable to chemical interactions with the soil. The other treatments, in general, gave lower results for water extractable calcium and magnesium than did the untreated cropped soil, which taken alone, might suggest that great absorption by the plants had depressed the amounts of calcium and magnesium capable of being extracted by water. It should be noted, however, that the depression of these elements so determined was generally and substantially lower in the case of those treatments which included phosphate, than in that of the nitrate treatment, although in one soil (No. 21), the nitrate treated portion

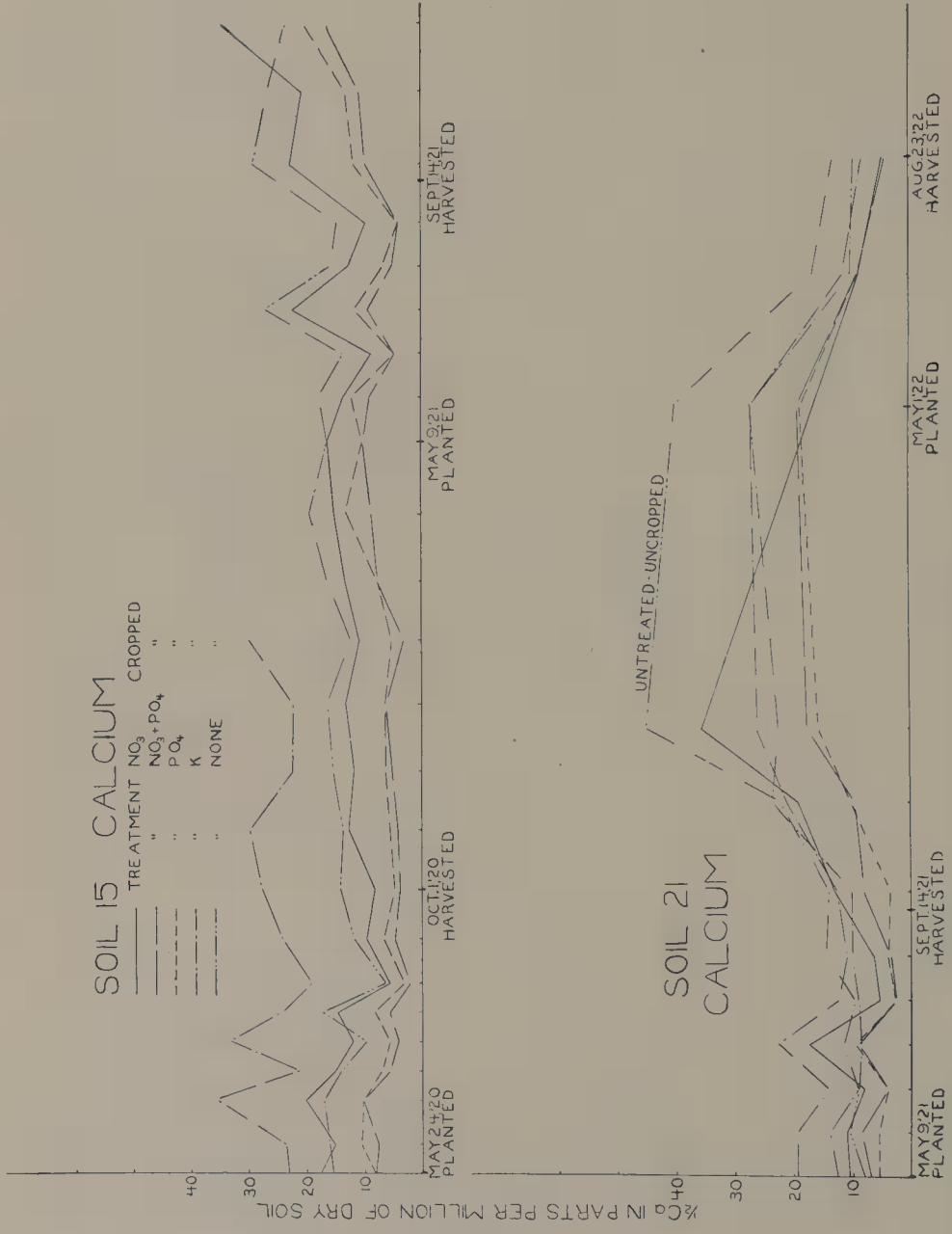


Fig. 4

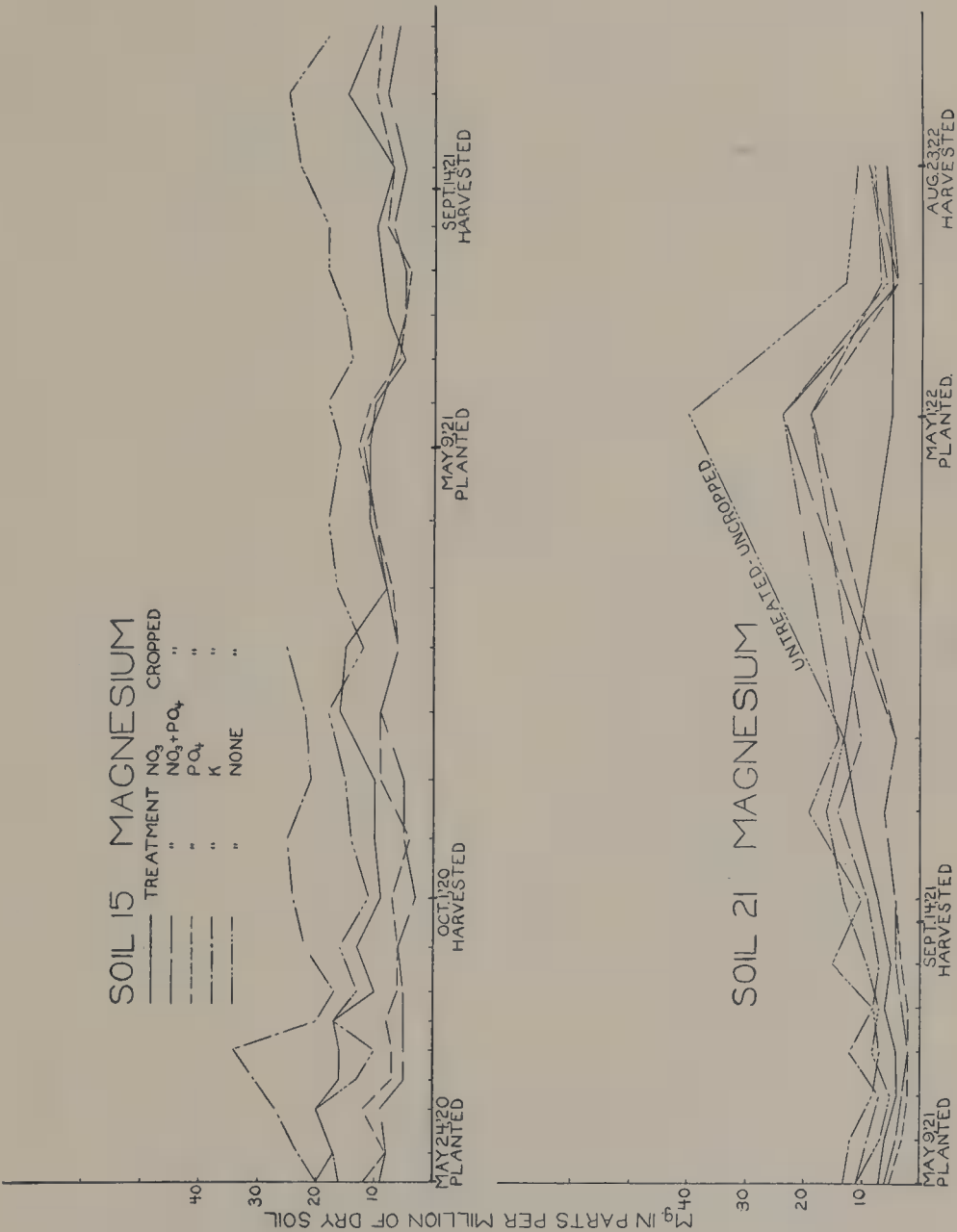


Fig. 5

yielded crops which absorbed greater absolute amounts of calcium and magnesium than did those receiving phosphate alone (see table 3B). This inconsistency and the small absolute values for calcium and magnesium withdrawn by the crops from both soils render it probable that interactions of calcium and magnesium with added phosphate have been the principal cause of the depression noted above. It is difficult to avoid the conclusion that a similar correlation must have existed in the true soil solution and that there is a very definite reciprocal relation between phosphate concentration and the amounts of calcium and magnesium in the liquid phase.

#### RELATION OF PLANT WITHDRAWALS TO CHANGES IN WATER EXTRACTABLE CONSTITUENTS

That a given element may be absorbed in greater quantity by a large and strong plant than by a less developed one upon a common substratum of soil or solution is generally recognized. It is also well known that if greater growth is obtained by the removal of some limitation, such as nitrogen deficiency, the plant usually absorbs larger quantities of other elements which do not themselves constitute limiting factors. It is evident that the causes of increased absorption cannot be dissociated where changes are brought about in the soil by addition of solutes or by other means, unless the crops obtained are relatively uniform in total yield of plant material. This result, obviously, cannot be brought about unless the limiting factor is one such as sunlight, which is extraneous to the soil itself. These considerations appear to require that we use a fertile soil in attempts to correlate plant withdrawals with changes in solubility of soil constituents.

Soil No. 15, considered above in relation to the changes brought about by additions of solutes, answers this requirement and when cropped actually did give relatively constant yields in seven, out of eight, different treatments. The one exception in which there was what appears to be a substantial increase in yield, due to changes in the solubility of soil constituents, was that in which both nitrate and phosphate were added to the soil, but no single so-called fertilizer constituent had that effect.



TABLE 3A  
WITHDRAWALS OF IMPORTANT CONSTITUENTS FROM VARIOUSLY TREATED SOIL BY 105 BARLEY PLANTS  
(Figures represent grams per tank)\*  
Fertile Soil No. 15. Season of 1920

Tank No.	Treatment Grams per tank	**Effect of treatment on water extractable constituents of the soil	Yield water-free dry matter	Constituents withdrawn				
				Nitrogen (N)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)
1	Nitrate of soda, 16 NO <sub>3</sub> .....	+NO <sub>3</sub> , = PO <sub>4</sub> , = Ca, = Mg, = K.....	800	12.65	3.67	11.55	2.81	1.55
2	Nitrate of soda, 33 NO <sub>3</sub> .....	+NO <sub>3</sub> , = PO <sub>4</sub> , = Ca, = Mg, = K.....	862	14.44	3.86	12.52	3.52	1.76
4	Untreated.....	None.....	786	11.16	3.34	10.21	2.42	1.33
5	Sodium-Hydrogen-Phosphate 162 PO <sub>4</sub> .....	= NO <sub>3</sub> , + PO <sub>4</sub> , - Ca, - Mg, = K.....	877	9.81	5.20	9.05	3.03	1.19
6	Sodium-Hydrogen-Phosphate 81 PO <sub>4</sub> .....	= NO <sub>3</sub> , + PO <sub>4</sub> , - Ca, - Mg, = K.....	894	10.43	4.00	9.59	2.09	1.05
7	Potassium chloride, 82 K.....	= NO <sub>3</sub> , = PO <sub>4</sub> , + Ca, + Mg, + K.....	792	10.80	3.34	12.56	2.78	1.30
8	Potassium chloride, 41 K.....	= NO <sub>3</sub> , = PO <sub>4</sub> , + Ca, + Mg, + K.....	818	10.26	3.21	12.74	2.10	1.28
3	Nitrate of soda, 33 NO <sub>3</sub> Sodium-Hydrogen-Phosphate 162 PO <sub>4</sub> .....	+NO <sub>3</sub> , + PO <sub>4</sub> , - Ca, - Mg, = K.....	1043	15.54	5.52	9.24	2.97	1.64

\*\* + means increase of water extractable constituents due to the treatment.

- means decrease of water extractable constituents due to the treatment.

= means no substantial change of water extractable constituents due to the treatment.

† These estimates are based upon behavior during the early part of the growing season and before the withdrawals by the crop had an opportunity to affect the results.

\* Figures for treatments and withdrawals may be converted into terms of P. P. M. of soil by multiplying by 1.23  $\left(\frac{1,000,000}{1800 \times 453}\right)$ , since each tank contained 1800 pounds of soil.

If we exclude from present consideration the data obtained from the soil in tank 3, nitrate-phosphate application (table 3A), the average yield of water-free dry matter is 833, the maximum is 894, and the minimum 786, grams per tank. No statistical study of the weights of individual plants was made, but these small variations in total yield probably fall within the limits of variability in the plants.

When we come to examine the absolute amounts of individual constituents withdrawn from each tank, it is by no means clear that many of the small differences observed represent significant variations, due to treatment. Another difficulty presents itself in that, while the water extractable constituents probably represent the soil solution, they are not a very precise measure of its concentration for constituents other than nitrate. For these reasons, only the most definite and apparently consistent relations between the effects of treatment and plant withdrawals will be noted.

#### COMPARISON OF WITHDRAWALS WITH EFFECTS OF TREATMENT WHEN NO INCREASE IN YIELD WAS OBTAINED

(Table 3A, excluding data from tank No. 3)

*Nitrate.*—In both cases where water extractable nitrate was increased in the soil, the withdrawal of nitrogen by the plant was increased more than with any other treatment, but without substantial increase in yield. Even if the differences in yield are regarded as significant, the nitrogen withdrawal is more than proportional thereto and the increased absorption of this element may thus be ascribed with a considerable degree of assurance to the increase of nitrate in the soil and not to a larger growth of the plants.

*Phosphorus.*—In both cases, where the water extractable phosphate of the soil was increased, the withdrawal of phosphorus was increased. If the differences in yield of crop are regarded as significant, the withdrawal of phosphorus is more than proportional thereto and apparently reflects the effect of the treatment on the soil. In two other cases (tanks 1 and 2), however, the phosphorus withdrawal is also somewhat greater than that from the untreated soil and slightly more than proportional to the actual differences in yield. No increase in water extractable phosphate has been brought about by these treatments and the correlation fails. It would appear either that the

difference observed is not significant or that phosphorus withdrawal from the portions of soil treated with sodium nitrate has been facilitated by changes in the soil solution which are not made evident by the present data.

*Potassium.*—The potassium withdrawal by plants in both cases of soil treated with potassium chlorid was greater than that from untreated soil, and may be reasonably ascribed to the increased amounts of water extractable potassium observed in the soil. It is also to be noted, however, that the same effect was produced by sodium nitrate treatment without a corresponding increase in water extractable potassium of the soil. The well known power of sodium to replace potassium in the solid phase suggests that there may have been a slight increase of potassium concentration in the soil solution which is not definitely reflected in the water extracts. On the other hand, the phosphate treatments involving much larger additions of sodium to the soil than was the case in the nitrate treatments, not only did not increase but actually decreased the potassium withdrawals. One explanation of this apparent anomaly is suggested by the fact that in the phosphate treatments the water extractable calcium and magnesium were lowered, and it is not impossible that potassium in the true soil solution has been decreased, although the effect is not definitely demonstrated by water extraction of the soil upon which these crops were grown. Another possible cause is suggested by recent work<sup>4</sup> in which it has been shown that increased sodium concentrations diminish the absorption of potassium from culture solutions.

*Calcium and magnesium.*—No uniformly consistent relation was shown between increases or decreases in water extractable calcium and magnesium, and withdrawals by the plant. Increased withdrawals of calcium were found when the water extractable calcium of the soil remained substantially unchanged and when it was slightly increased or diminished. Decreased withdrawals were also found when the water extractable calcium was slightly increased, and when it was somewhat diminished by the treatments. Magnesium shows increased withdrawals when the water extractable magnesium remained substantially unchanged, and diminished withdrawals when the water extractable magnesium was slightly diminished. In two cases, a slight increase in water extractable magnesium was noted without apparent effect upon withdrawal.

COMPARISON OF WITHDRAWALS WHEN AN INCREASED YIELD  
WAS OBTAINED

When an increase in yield was obtained (table 3A, cf. tanks 3 and 4), there was an increase in withdrawal of all constituents, except potassium. The increased withdrawal of nitrogen and phosphorus is clearly referable to the large increases in water extractable nitrate and phosphate. The water extractable calcium and magnesium have been diminished by the treatment, but the increased withdrawals of these constituents can be accounted for by the increased size and absorbing power of the crop. The diminished absorption of potassium by the larger crop must be accounted for by such reasons as have been suggested heretofore in the consideration of data from the crops where no increases in yield were obtained.

When we turn to the results from an infertile soil, it is at once evident that the withdrawal of soil constituents as a whole was more closely correlated with the yields than with changes in amount of water extractable constituents. Thus increased yields have enabled the plant to take up significantly larger quantities of calcium and magnesium when these were neither added to the soil nor increased in concentration by indirect methods as indicated by water extraction. Moreover, instances occur where substantial increases in withdrawal of the elements occur with diminution of water extractable calcium and magnesium. In the case of potassium, there are two instances where larger quantities of this element are removed from the soil when the water extractable potassium remained relatively constant than when large amounts of potassium chlorid were added to the soil and a very great increase in water extractable potassium was developed.

In all cases where increased yields were obtained, phosphorus withdrawal was substantially greater than that from the untreated portion of soil. When a large yield was obtained from soil in which no increase of water extractable phosphate was induced by the treatment (tank 1), there was as much phosphorus withdrawn from the soil as where a substantial increase of water extractable phosphate was brought about by treatment (tank 4), but where the yield of crop was not so great. The only clear correlation between withdrawals and the amounts of water extractable constituents in this soil is in the case of nitrogen. It is evident that the soil under discussion is one which

TABLE 3B  
WITHDRAWALS OF IMPORTANT CONSTITUENTS FROM VARIOUSLY TREATED SOIL BY 105 BARLEY PLANTS  
(Figures represent grams per tank)\*  
Infertile Soil No. 21. Season of 1921

Tank No.	Treatment Grams per tank	**Effect of treatment on water extractable constituents of the soil	Yield water-free dry matter	Constituents withdrawn				
				Nitrogen (N)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)
3	Untreated.....	None.....	306	4.63	1.21	4.59	1.31	0.48
5	Potassium chloride, 81 K.....	+NO <sub>3</sub> , = PO <sub>4</sub> , +Ca, = Mg, +K.....	628	6.10	2.18	9.68	2.28	1.12
4	Sodium-Hydrogen-Phosphate 101 PO <sub>4</sub> .....	+NO <sub>3</sub> , +PO <sub>4</sub> , -Ca, -Mg, -K.....	630	6.32	3.76	6.62	2.10	0.95
1	Nitrate of soda, 82 NO <sub>3</sub> .....	+NO <sub>3</sub> , = PO <sub>4</sub> , = Ca, -Mg, = K.....	1035	16.45	3.82	13.27	3.69	1.34
2	Nitrate of soda, 82 NO <sub>3</sub> , Sodium-Hydrogen-Phosphate, 101 PO <sub>4</sub> .....	+NO <sub>3</sub> , +PO <sub>4</sub> , -Ca, -Mg, -K.....	1144	18.40	6.25	12.58	3.85	1.54

\*\*+means increase of water extractable constituents due to the treatment.

-means decrease of water extractable constituents due to the treatment.

=means no substantial change of water extractable constituents due to the treatment.

These estimates are based upon behavior during the early part of the growing season and before the withdrawals by the crop had an opportunity to affect the results.

\*Figures for treatments and withdrawals may be converted into terms of P. P. M. of soil by multiplying by 1.23  $\left(\frac{1,000,000}{1800 \times 453}\right)$ , since each tank contained 1800 pounds of soil.



is deficient in power to supply nitrogen to the plant, but that almost any salt treatment stimulates nitrification, a result which is reflected in the yield of the crop, and in the power of the crop to acquire other elements even though these latter are diminished in solubility in the soil. This lack of correlation between withdrawals of elements which are not primarily limiting growth and the solubility of these same elements in the soil can hardly be charged to the inadequacy of water extraction as a criterion of that solubility. Probably no one will insist that diminished figures for water extractable constituents connote an increase in concentration of such constituents in the soil solution; yet such diminished figures frequently appear concurrently with increased withdrawals. The cause of increased withdrawals in such cases is to be sought in the greater total absorbing power of the plants due to a larger growth. We do not suggest that withdrawals of constituents which appear to be quantitatively of secondary importance in growth, are not a factor in growth; but it would appear that the amount of growth, however produced, is a preponderating element in causing an increased withdrawal of such constituents from the soil.

#### EFFECTS OF TREATMENTS AND WITHDRAWALS UPON SUBSEQUENT CROPS

The data covering yields of crops the second season after treatment have been presented (see tables 2A and 2B). Unfortunately, as stated in the footnote to table 2A, the second year's crop on soil No. 15 was attacked by a fungous disease which vitiates the data for that year. The effect of the first year's treatment and withdrawals on the second year's yield is shown by the data from soil No. 21 (table 2B). Here, it will be observed, there was a falling off in yield from all plots of soil whatever the treatment. The yield of the untreated soil has fallen off consistently with its known infertile character. One of the plots (tank 4), which gave a fairly large yield the first year under the influence of treatment, apparently suffered the second year because of the increased withdrawals by the preceding crop. The plots which received nitrate and produced large crops the first year retained their relative superiority the second year, but the amount of crop then produced was probably not significantly greater than that of the untreated plot the first year. When an infertile soil produces such a small absolute yield, it is dangerous to draw inferences from what appear to



be considerable differences in relative yield. The most that can be said under the circumstances is that the greater withdrawals from tanks 1 and 2 the first year have not prevented a relatively good yield the second year (as compared with the untreated plot). It will be recalled, however (fig. 1), that differences in nitrate content of all of the soil disappear the first year and it is evident that the superiority of the nitrate treated plots the second year, if regarded as significant, is not due to any residuum of nitrate remaining in the soil, but to indirect effects of the reaction of the salt sodium nitrate on the soil.

### SUMMARY

1. Two soils treated with various solutions and salt mixtures, when examined after eight months, showed substantial increases in water extractable constituents in general. In most cases, the increase of a given constituent was less than the amount of solute added, but magnesium and sulfate in one of the two soils increased more than could be accounted for by the addition of these elements.

2. The changes induced are ascribed to added solutes, to chemical replacements of solid phase material, to fixation by the solid phase, and to increased solubility of solid phase material in the new soil solution.

3. Two soils upon which crops were grown received various treatments of nitrate of soda, sodium dihydrogen phosphate, and potassium chlorid. Increase of the added solute was always observed in water extracts of the soil in the early part of the season. Additions of nitrate, however, did not prevent the practical disappearance of this ion at the height of the growing season. A reciprocal relation between added phosphate and other solutes is made evident, there being diminished amounts of water extractable calcium and magnesium in both soils, and of potassium in one soil when the water extractable phosphate was increased by treatment.

4. When no increase of crop was brought about by treatment of the soil, a correlation was observed between increases in given constituents in the soil as measured by water extraction (1-5), and the withdrawals of such constituents by the plants. On the other hand, increased or diminished withdrawals were frequently observed which bore no apparent relation to increased or diminished amounts of water

extractable constituents. This result may be ascribed to the defects of water extraction as a measure of the soil solution or to the influence of changed concentrations of other constituents upon the absorption of a given element by the plant.

5. When an increase of crop was brought about by treatment of the soil, there was a definite correlation between withdrawals of some constituent, or constituents (nitrate and phosphate in soil 15, nitrate in soil 21), and the yields of dry matter obtained; but the amounts of other constituents withdrawn appear to have been determined either by changed concentrations, or by the increased amounts of dry matter produced.

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THE RESPIRATION OF POTATO TUBERS  
IN RELATION TO THE OCCURRENCE  
OF BLACKHEART

BY

J. P. BENNETT AND E. T. BARTHOLOMEW

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INTRODUCTION

The artificial production of blackheart in potato tubers was accomplished by Bartholomew<sup>3</sup> in 1915. He found that exposing potatoes to temperatures of 38° to 48° C. with free access of air, for 15 to 20 hours resulted in the subsequent blackening of the interior regions of the tuber after removal to ordinary temperatures. Most marked results were obtained by exposure between 42° and 44° C., and this range was at that time designated as the optimum temperature for the production of this pathological condition.

Bartholomew ascribed the production of the injury to a deficiency of oxygen in the injured regions of the tuber, brought about as a result of the increased rate of respiration of the tissues. Under these conditions the available supply of oxygen diffusing inward from the surface of the tuber was used up before it reached the interior tissues which died as a result of asphyxiation. When oxygen was allowed to penetrate to these tissues later, they blackened in consequence of the action of tyrosinase upon the tyrosin which is normally present in the uninjured tuber and in increased amount in the injured regions.

That the oxygen supply was of first importance to the production of blackheart in heated tubers was confirmed by other experiments of Bartholomew in which the external supply of oxygen was varied, or its penetration into the interior tissues of the tuber favored. It was found

that passing a stream of approximately 94% oxygen over the tubers during the period of heating prevented the development of blackheart. On the other hand, passing a stream of approximately 94% carbon dioxide over the tubers markedly increased the severity of the injury. Passing a stream of ordinary air over the tubers during the period of heating had little effect in comparison with tubers heated in still air. It was learned subsequently that partly or completely peeling the tubers, or abrading their surfaces by hacking with a knife usually lessened or prevented injury. The time of heating required to produce blackheart in tubers thus treated was longer than when the skin was left intact, the injury was less severe or did not occur within the usual period of heating. It was also noticed that large tubers were more susceptible to injury than small ones.

At the time Bartholomew published his results he did not suspect that blackheart could be produced at temperatures lower than 38° C., although oxygen supply was shown to be the prime factor in its production. It was shown by Stewart and Mix<sup>15</sup> in 1917 that blackheart could be produced readily at low temperatures by restricting the supply of oxygen sufficiently. Using jars and tanks entirely or partly filled with tubers, sealed air-tight and placed at various temperatures ranging from 2° to 24° C., for periods ranging from a few days to 58 days, they were able to produce typical blackheart. With partly filled containers, or lower temperatures, longer exposure was required to produce the injury than with filled containers or higher temperatures. With a volume of air equal to that of the tubers in the containers an exposure of 10–12 days at about 21° C., of about 20 days at 12°–16° C., and of 23–40 days at about 5° C., was required to produce blackheart. With a larger proportion of air to tubers a longer exposure was required. When the ratio of volume of air to volume of tubers was 26.9 or greater no blackheart was produced in 40 days at a temperature of 15°–22° C.

It was further shown by Stewart and Mix that deficient oxygen supply and not the accumulation of carbon dioxide resulting from the respiration processes was directly responsible for the injury. In experiments with tubers in sealed containers, into some of which were placed solutions of sodium hydroxide sufficient to absorb the carbon dioxide produced, blackheart occurred about equally severely in all jars.

Mann and Joshi,<sup>9</sup> in India, found that tubers heated in open wire baskets in ordinary air at a temperature of 27°–30° C., remained sound for periods longer than twelve days. When heated at 36° C., under the same conditions of aeration, blackheart occurred in about six days. At 41°–42° C., the injury was very marked in two days. When the tubers were placed in sealed containers in which the air was replaced by carbon dioxide or nitrogen, or when the tubers were coated with collodion or paraffin, blackheart was found to develop at 27°–30° C., in six to twelve days. At 36° C., injury had occurred on the third day. These results are in agreement with the observations of Bartholomew, except that he did not find the occurrence of blackheart at a temperature lower than 38° C. Tubers exposed by Bartholomew at 32°–33° C., were uninjured after 16 days. These tubers, however, were placed in the oven only at night and left at laboratory temperatures during the day. Exposure for two successive nights at 38°–39° C., with removal to laboratory temperatures during the day, resulted in the appearance of blackheart in some of the tubers. Later observations by the writers showed that continuous heating occasionally produced blackheart at temperatures as low as 25° C.

It is evident from the work of these investigators that there is a relationship between temperature, oxygen supply, and period of exposure in the production of blackheart both at the low and high ranges of temperature used. Increased temperature and reduced supply of oxygen apparently decrease the period of exposure required to induce blackheart.

The gaseous exchange between the tubers and the surrounding atmosphere has not previously been followed, and the conditions obtaining at the time when injury appears are inadequately known, except in those cases where blackheart has been produced by heating the tubers in normal air. It is the purpose of this paper to report a more extended study of the relationship of temperature, time, and oxygen supply in the production of blackheart. Such a study appears desirable, since blackheart has been reported to occur not only during shipment in heated cars, but during storage, and in the field, especially in the western states. Its occurrence in the field has been reported from six counties in interior valleys of central and southern California. Its occurrence in the field in Utah has been reported to one of the writers by Dr. B. L. Richards of the Utah Experiment Station, and in Idaho

by Dr. G. K. K. Link of the U. S. Dept. of Agriculture. A reputed case of considerable loss due to blackheart in cold storage was brought to the writers' attention in San Francisco in 1920.\*

### METHODS

Measured volumes of tubers were sealed in containers of known capacity so as to provide a definite ratio of air volume to volume of tubers. Each series, consisting of five to eleven containers, was then held at a uniform temperature. At successive intervals one or more containers were removed, samples of the atmosphere withdrawn and analyzed. The containers were then opened and the condition of the tubers observed. Each series was kept at a given temperature until blackheart appeared in the tubers removed at two or more successive times.

The containers used were glass jars of approximately 5½ liters capacity, with ground lids pierced by two holes closed with glass stopcocks. To the inner end of one stopcock was attached a rubber tube which extended to the bottom of the jar. The tubers were supported near the middle of the jar on an inverted wire basket. The sealed jars were tested for air-tightness by introducing compressed air and immersing in water. After placing the jars in the incubator or cold storage room the stopcocks were left open, or opened at frequent intervals, until the jars and contents had reached the temperature of their surroundings. The stopcocks were then closed and remained so until removal of the jar. On removing each jar any change of pressure of the atmosphere within was determined by means of a manometer. The atmosphere within the jar was then thoroughly mixed by agitation, and two samples withdrawn, one from the upper and the other from the lower part of the jar. Before opening, the jar was again tested for air-tightness.

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\* The work reported here was begun in 1918 at the University of Wisconsin for the U. S. Dept. of Agriculture, Bureau of Plant Industry. Since 1919 the work has been carried on at the University of California at Berkeley. Acknowledgment is due to the Botany Department of the University of Wisconsin for certain facilities, to the Office of Cotton, Truck, and Forage Crop Disease Investigations, U. S. Dept. of Agriculture for support during the early part of the work and to the Division of Agronomy, College of Agriculture, for the tubers used in the work at Berkeley.



The duplicate gas samples were analyzed for oxygen and carbon dioxide over mercury by means of a Hempel or an Orsat apparatus, readings being made to two-tenths of one per cent. The results of analyses are in all cases expressed as per cent of volume.

The tubers used in the work at Wisconsin were of the varieties Rural New Yorker and Green Mountain Jr., obtained from a local seedsman and stored in a cellar at a temperature of 10°–15° C. previous to use. The experiments were begun in November so that the tubers which were locally grown were past or very nearly past their normal rest period when first used. The tubers used in the work at Berkeley were of the variety Netted Gem, grown in Nevada, purchased on the local market in October, and stored at 5° C. until used. Experiments were begun in December so that these tubers were also past their normal rest period at the time of use. The volumes of potatoes placed in the jars were calculated from their specific gravity which was found to average approximately 1.1. The ratio of volume of air to volume of tubers in each jar varied in the work in Wisconsin, from 5.8 to 7.0; in the work at Berkeley the ratio was approximately 6. The tubers were weighed to within 5 grams of the calculated required weight. The number of tubers in each jar varied from 5 to 7 in all but a few instances.

The intervals between removals of jars in a given series varied from one hour at the highest temperature to one week at the lowest temperature. On removal of the tubers after exposure they were split longitudinally and exposed to the laboratory air for several hours before observations were made. In recording the condition of the tubers three stages of severity of injury were recognized: "slight," denoting small injured areas of less than one-tenth the area of the exposed cut surface of the tuber; "moderate," indicating an injured area amounting to between one-tenth and one-third of the cut surface; and "severe," applying to those showing still greater extent of injury. No attempt was made to determine the actual volume of tissue injured, but on the basis just noted it would correspond roughly to less than 0.1 per cent of the tuber volume for slight, 0.1 to 4 per cent for moderate, and over 4 per cent for severe injury. In each series the end of the period required for the development of blackheart was determined by the first jar in which blackheart appeared in one or more tubers when

followed by its appearance in the succeeding withdrawals. The series usually extended beyond this point, acting thus as a check upon the first observations.

The incubators and cold storage rooms utilized in these experiments were automatically regulated to within  $1^{\circ}$  C. The experiments were conducted at temperatures ranging from  $0^{\circ}$  to  $45^{\circ}$  C., the intervals being two and a half degrees between  $0^{\circ}$  and  $10^{\circ}$  C., and five degrees between  $10^{\circ}$  and  $45^{\circ}$  C.

#### DATA

Table 1 presents the data obtained in Wisconsin with Rural New Yorker and Green Mountain Jr. potatoes. Control experiments were conducted in parallel with those in sealed jars, with an equal number of tubers from the same lot placed in wire baskets.

In table 1, and in all succeeding tables, all values in horizontal columns referring to gas content of jars are expressed as per cent of volume. Since the ratio of volume of gas to volume of tubers was approximately the same in all jars, the values for the content of carbon dioxide and oxygen in the different jars may be compared directly. No marked changes in pressure of the gases in the jars occurred in these experiments. In the horizontal columns referring to the condition of tubers the sum of the numbers in each section indicates the total number of tubers in the jar opened at that point of the series. The signs refer to presence or absence of blackheart as follows: — indicates that no blackheart had developed; + indicates slight; ++ moderate; and +++ severe injury with corresponding development of blackheart, as described for these designations in the preceding section of this paper.



TABLE I

GASEOUS EXCHANGE AND THE PRODUCTION OF BLACKHEART IN RURAL NEW YORKER AND GREEN MOUNTAIN JR. POTATO TUBERS WHEN  
SUBJECTED TO DIFFERENT TEMPERATURES IN SEALED CHAMBERS

Temperature .....	Rural New Yorker tubers						Green Mountain Jr. tubers					
	40° C.						40° C.					
Time, days.....	0	1/6	1/3	1/2	1	.....	0	1/6	1/3	1/2	1	.....
CO <sub>2</sub> evolved %.....	0	1.2	3.4	5.4	9.2	.....	0	1.6	4.2	6.6	11.2	.....
O <sub>2</sub> remaining %.....	21	19.8	17.4	15.2	11.6	.....	21	19.2	16.6	14.2	9.2	.....
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	21.0	20.8	20.6	20.8	.....	21	20.8	20.8	20.8	20.4	.....
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed	.....	1.0	0.94	0.95	0.98	.....	.....	0.89	0.95	0.97	0.95	.....
Blackheart in tubers in jars.....	6-	5-	6+	1+	.....	.....	6-	4-	1-	2++	.....	.....
		1+		4++				2+	3+	4++		
				1++					2++			
Blackheart in control tubers.....	6-	5-	4-	1-	.....	.....	6-	6-	3-	1+	.....	.....
		1+	2+	4++					3+	3++		
				1++					3+	2++		
Temperature.....	35° C.						35° C.					
	35° C.						35° C.					
Time, days.....	0	1/3	2/3	1	1 1/3	.....	0	1/3	2/3	1	1 1/3	.....
CO <sub>2</sub> evolved %.....	0	2.2	4.8	7.6	9.6	.....	0	2.6	6.0	9.6	12.2	.....
O <sub>2</sub> remaining %.....	21	18.4	15.8	13.0	10.4	.....	21	18.2	14.2	10.4	7.2	.....
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	20.6	20.6	20.6	20.0	.....	21	20.8	20.2	20.0	19.4	.....
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed	.....	0.85	0.92	0.95	0.91	.....	.....	0.93	0.88	0.91	0.88	.....
Blackheart in tubers in jars.....	.....	6-	5-	5-	6+	.....	.....	8-	3-	3-	2-	.....
			1+	1+				1+	4+	3+	4++	
										3++	4++	
Blackheart in control tubers.....	.....	6-	6-	6-	4-	.....	.....	9-	4-	4-	3-	.....
									3+	5+	5+	
					2+					2++	2++	

TABLE 1—(Continued)

Rural New Yorker tubers										Green Mountain Jr. tubers									
30° C.										30° C.									
Temperature.....										Temperature.....									
Time, days.....	0	1	2	3	4					0	1	2	3	4					
CO <sub>2</sub> evolved %.....	0	6.4	10.4	14.2	18.0					0	7.8	12.4	16.6	22.4					
O <sub>2</sub> remaining %.....	21	14.2	9.2	5.8	2.0					21	13.0	7.8	3.2	0.4					
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	20.6	19.6	20.0	20.0					21	20.8	20.2	19.8	22.8					
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed.....		0.94	0.83	0.93	0.95						0.97	0.94	0.93	1.09					
Blackheart in tubers in jars.....		6—	5—	3—	2+						5—	3—	1+	6+++					
				2+	4++						1+	3+	3++						
Blackheart in control tubers.....		6—	5—	5—	6—						6—	5—	6—	4—					
												1+	2++	2+					
25° C.										25° C.									
Temperature.....										Temperature.....									
Time, days.....	0	1	2	3	4	5	6			0	1	2	3	4	5	6			
CO <sub>2</sub> evolved %.....	0	4.6	8.0	10.8	12.4	14.8	16.6			0	6.4	10.6	14.0	16.8	19.2	20.0			
O <sub>2</sub> remaining %.....	21	15.6	12.2	8.8	6.6	4.2	2.8			21	14.4	9.8	6.0	3.0	1.2	0.8			
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	20.2	20.2	19.6	19.0	19.0	19.4			21	20.8	20.4	20.0	19.8	19.4	20.8			
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed.....		0.85	0.91	0.89	0.86	0.83	0.91				0.97	0.95	0.93	0.93	0.92	0.98			
Blackheart in tubers in jars.....		7—	7—	7—	7—	6—	2—				6—	6—	6—	5—	4—	2—			
						1+	4++							1+	2+	4+			
Blackheart in control tubers.....		7—	7—	7—	7—	7—	7—				6—	6—	6—	6—	6—	4—			
																2+			

TABLE 1—(Concluded)

	Rural New Yorker tubers							Green Mountain Jr. tubers						
Temperature.....	20° C.							20° C.						
Time, days.....	0	1	3	5	7	9	11	0	1	3	5	7	9	11
CO <sub>2</sub> evolved % .....	0	2.6	7.4	10.2	13.2	15.2	17.2	0	3.4	10.4	13.2	15.6	21.0	23.4
O <sub>2</sub> remaining % .....	21	18.2	11.4	8.4	4.6	2.8	1.4	21	17.6	9.8	7.0	4.0	1.0	0
CO <sub>2</sub> +O <sub>2</sub> present % .....	21	20.8	18.8	18.6	17.8	18.0	18.6	21	21.0	20.2	20.2	19.6	22.0	23.4
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed	.....	0.93	0.77	0.81	0.81	0.84	0.88	.....	1.0	0.93	0.94	0.92	1.05	1.11
Blackheart in tubers in jars.....	.....	7—	7—	7—	7—	6—	2—	.....	8—	8—	7—	7—	6—	2—
						1+	5+						1+	4+
Blackheart in control tubers.....	.....	7—	7—	7—	7—	7—	7—	.....	8—	8—	7—	7—	7—	1++ 7—

TABLE 2  
GASEOUS EXCHANGE AND THE PRODUCTION OF BLACKHEART IN NETTED GEM POTATO TUBERS WHEN SUBJECTED TO DIFFERENT TEMPERATURES IN SEALED CHAMBERS

Temperature.....		45° C.									
Time, days.....	0	2/24	3/24	4/24	5/24	6/24	8/24				
CO <sub>2</sub> evolved %.....	0	1.8	2.4	3.6	4.8	5.2	5.2				
O <sub>2</sub> remaining %.....	21	18.2	18.0	16.6	15.4	15.0	14.6				
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	20.0	20.4	20.2	20.2	20.2	19.8				
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed.....		0.63	0.80	0.82	0.86	0.87	0.81				
Blackheart in tubers.....		5-	5-	3-	4-	4-	1-				
				2+	1+	1++	4++				
Temperature.....		40° C.									
Time, days.....	0	4/24	6/24	7/24	8/24	9/24	10/24	12/24	18.5 24	1	1½
CO <sub>2</sub> evolved %.....	0	2.8	4.4	5.0	6.0	6.8	7.0	7.8	12.2	15.0	21.6
O <sub>2</sub> remaining %.....	21	17.2	15.6	15.2	14.2	12.8	13.2	12.2	9.2	7.2	2.8
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	20.0	20.0	20.2	20.2	19.6	20.2	20.0	21.4	22.2	24.4
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed.....		0.74	0.81	0.86	0.88	0.83	0.90	0.89	1.13	1.09	1.18
Blackheart in tubers.....		5-	5-	3-	2-	1-	2+	1+	3++	1+	5+++
				3+	3+	4++	3++	4++	3+++	4+++	
					2++						

TABLE 2—(Continued)

35° C.										
Temperature.....										
Time, days.....	0	2	3	3½	4	5				
CO <sub>2</sub> evolved %.....	0	13.0	15.8	20.0	24.8	25.4				
O <sub>2</sub> remaining %.....	21	5.0	3.0	1.0	0.4	0				
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	18.0	18.8	21.0	25.2	25.4				
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed.....		0.81	0.88	1.0	1.2	1.2				
Blackheart in tubers.....		5—	4— 1+	3— 1+	1+ 3+++	1+ 3+++				

30° C.										
Temperature.....										
Time, days.....	0	0.5	1	1.5	2	2.5	3	4	5	6
CO <sub>2</sub> evolved %.....	0	2.0	6.0	8.0	10.2	12.6	13.0	16.6	21.0	22.2
O <sub>2</sub> remaining %.....	21	17.4	13.4	11.2	8.8	6.4	5.8	2.2	0	0
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	19.4	19.4	19.2	19.0	19.0	18.8	18.8	21.0	22.2
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed.....		0.56	0.79	0.82	0.84	0.86	0.86	0.88	1.00	1.06
Blackheart in tubers.....		5—	5—	4—	5—	5—	5—	5—	2— 3+	1— 3+ 1++

TABLE 2—(Continued)

25° C.										
Temperature.....	0	1	2	3	4	5	6	7	8	9
Time, days.....	0	4.4	9.4	11.8	15.2	15.4	18.4	20.4	21.0	23.0
CO <sub>2</sub> evolved %.....	0	14.8	10.0	7.0	3.6	3.2	0.8	0	0	0
O <sub>2</sub> remaining %.....	21	19.2	19.4	18.8	18.8	18.6	19.2	20.4	21.0	23.0
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	0.71	0.85	0.84	0.87	0.86	0.91	0.97	1.00	1.10
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed }.....										
Blackheart in tubers.....		5—	5—	5—	5—	5—	5—	2—	3+	3+
								3+	2++	2++

20° C.										
Temperature.....	0	2	4	6	8	10	12	13		
Time, days.....	0	7.0	11.8	15.4	16.2	20.4	23.4	21.4		
CO <sub>2</sub> evolved %.....	0	10.8	6.0	2.0	1.4	0	0	0		
O <sub>2</sub> remaining %.....	21	17.8	17.8	17.4	17.6	20.4	23.4	21.4		
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	0.69	0.79	0.81	0.83	0.97	1.11	1.02		
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed }.....										
Blackheart in tubers.....		5—	5—	5—	5—	5—	3—	5+		
							2+			



TABLE 2—(Continued)

15° C.												
Temperature.....												
Time, days.....	0	2	6	10	14	18	22	26	30	34	38	
CO <sub>2</sub> evolved %.....	0	2.8	8.8	10.8	13.8	15.0	18.0	22.0	20.8	30.8	28.6	
O <sub>2</sub> remaining %.....	21	16.0	9.2	5.6	3.4	2.0	0	0	0	0	0	
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	18.8	18.0	16.4	17.2	17.0	18.0	22.0	20.8	30.8	28.6	
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed }.....		0.56	0.75	0.70	0.78	0.79	0.86	1.05	0.99	1.47	1.36	
Blackheart in tubers.....		6—	5—	5—	5—	5—	5—	5—	5—	5+	2— 3+	

10° C.												
Temperature.....												
Time, days.....	0	7	14	21	28	33	38	42	46	50		
CO <sub>2</sub> evolved %.....	0	7.6	15.4	20.8	21.4	24.6	22.8	25.6	28.2	30.0		
O <sub>2</sub> remaining %.....	21	10.4	1.8	0	0	0	0	0	0	0		
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	18.0	17.2	20.8	21.4	24.6	22.8	25.6	28.2	30.0		
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed }.....		0.72	0.80	0.99	1.02	1.17	1.08	1.22	1.34	1.43		
Blackheart in tubers.....		6—	6—	7—	6—	5—	5—	2— 3+	3+	4++ 1++		

TABLE 2—(Continued)

Temperature.....	7.5° C.											
	0	8	16	22	29	35	44	50	57	64	73	78
Time, days.....	0	4.2	7.2	11.6	13.2	15.4	18.8	19.0	20.6	22.8	27.2	42.8
CO <sub>2</sub> evolved %.....	0	4.2	7.2	11.6	13.2	15.4	18.8	19.0	20.6	22.8	27.2	42.8
O <sub>2</sub> remaining %.....	21	14.0	6.4	3.4	1.0	0	0	0	0	0	0	0
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	18.2	13.6	15.0	14.2	15.4	18.8	19.0	20.6	22.8	27.2	42.8
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed }.....	0.60	0.49	0.66	0.66	0.66	0.73	0.90	0.91	0.98	1.09	1.3	2.0
Blackheart in tubers.....		6—	5—	5—	5—	5—	5—	4— 1++	3— 2++	1— 2+ 2++	1++ 4+++	1+ 4+++
	5° C.											
	0	7	14	21	28	35	42	49	56	70	77	84
Time, days.....	0	3.8	8.2	13.0	16.8	19.8	19.2	21.6	23.2	25.0	26.4	34.2
CO <sub>2</sub> evolved %.....	0	3.8	8.2	13.0	16.8	19.8	19.2	21.6	23.2	25.0	26.4	34.2
O <sub>2</sub> remaining %.....	21	14.4	9.8	3.6	0	0	0	0	0	0	0	0
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	18.2	18.0	16.6	16.8	19.8	19.2	21.6	23.2	25.0	26.4	34.2
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed }.....	0.58	0.73	0.75	0.75	0.80	0.94	0.92	1.03	1.10	1.19	1.26	1.63
Blackheart in tubers.....		5—	5—	5—	5—	5—	5—	5—	5—	5+*	5++	5++

\* Injury occurred on surface only; no injury appeared in interior of tubers. In two following jars injury occurred both on surface and in interior of tubers.

TABLE 2—(Concluded)

Temperature.....		2.5° C.									
Time, days.....	0	8	14	21	29	36	41	47	54	61	.....
CO <sub>2</sub> evolved %.....	0	3.2	6.4	9.6	15.4	19.0	22.4	22.8	21.4	24.8	.....
O <sub>2</sub> remaining %.....	21	14.0	10.0	5.0	1.0	0	0	0	0	0	.....
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	17.2	16.4	14.6	16.4	19.0	22.4	22.8	21.4	24.8	.....
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed }.....	.....	0.46	0.58	0.60	0.77	0.90	1.07	1.09	1.02	1.18	.....
Blackheart in tubers.....	.....	8—	5—	6—	6—	4— 2+	5—	4— 1+	4+ 2++	3++ 2++	.....

Temperature.....		0° C.									
Time, days.....	0	7	15	23	30	37	40	44	47	.....	.....
CO <sub>2</sub> evolved %.....	0	2.8	8.8	15.8	18.0	22.2	22.0	22.4	20.8	.....	.....
O <sub>2</sub> remaining %.....	21	16.2	8.4	0.8	0	0	0	0	0	.....	.....
CO <sub>2</sub> +O <sub>2</sub> present %.....	21	19.0	17.2	16.6	18.0	22.2	22.0	22.4	20.8	.....	.....
Ratio CO <sub>2</sub> evolved to O <sub>2</sub> absorbed }.....	.....	0.58	0.70	0.78	0.86	1.06	1.05	1.07	0.99	.....	.....
Blackheart in tubers.....	.....	5—	8—	8—	6—	5— 2+	7+ 1++	3+ 3++	4++ 2++	.....	.....

The studies were not carried further in Wisconsin because of the lack of controlled temperatures below 20° C. The data presented in table 2 were obtained at Berkeley where cold storage facilities were available in addition to incubators. Since Bartholomew<sup>3</sup> found differences in susceptibility to blackheart among different varieties it was necessary to repeat the experiments at the higher temperatures in the work at Berkeley. Control experiments were not conducted in any case at Berkeley, dependence being placed upon the continuity of each series, adjoining jars thus serving as checks.

In table 3, is presented a summary of the data given in detail in table 2, showing the results obtained with Netted Gem tubers. The calculations of average daily absorption of oxygen (column 7) were based upon the period between the beginning of the series and the point where total exhaustion of oxygen occurred in the series. In all other cases, except where noted, the values given are based upon the single jar removed at the time when the initial appearance of blackheart occurred. This jar was the culminating point of each series and represents fairly well the average condition in the series at that point. The values in columns 1, 3, 4, 5, 6 and 9 are shown graphically in figure 1.

Columns 10, 11 and 12 show the maximum effects of the unequal rates of absorption of oxygen and evolution of carbon dioxide upon the total amounts of these two gases in the atmospheres of the jars. The values for respiratory ratios in column 13 show the relative absorption of oxygen and evolution of carbon dioxide which had occurred up to this time.

In table 4 is shown the effect upon the rate of respiration of the exhaustion of the free oxygen from the atmosphere of the jars. The values given in all instances are the averages of the results from all the jars removed previous to the time of total disappearance of oxygen (middle horizontal column), and from all the jars removed between that point and the first appearance of blackheart in the series (lower horizontal column). Where widely varying or abnormally appearing averages are given the values from which these averages were obtained are given in parentheses. The abnormally low value for the period without free oxygen at 10° C. cannot be explained with the available data.



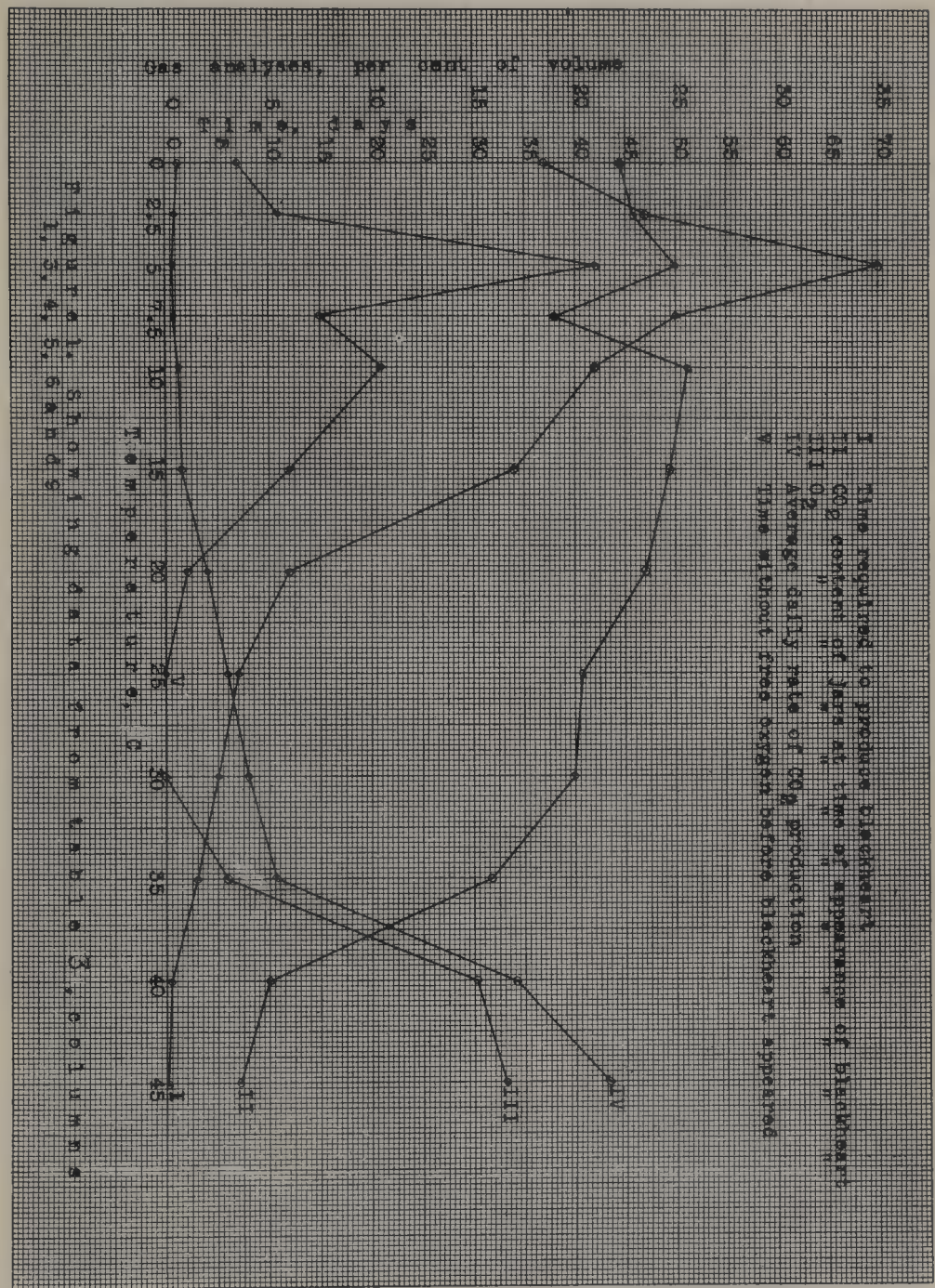


TABLE 3  
A SUMMARY OF THE MAIN RESULTS OF TABLE 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Temperature	Interval of observations	Period of exposure to produce black-heart	Analyses of gases in jar at time blackheart appeared		Aver. daily production of CO <sub>2</sub>	Aver. daily absorption of O <sub>2</sub>	Period for total absorption of O <sub>2</sub> in jar	Period without free O <sub>2</sub> before black-heart appeared	CO <sub>2</sub> +O <sub>2</sub> minimum in each series			Respiratory ratios		
			CO <sub>2</sub>	O <sub>2</sub>					CO <sub>2</sub> +O <sub>2</sub>	De-crease from 21%	Time of appearance after start	At minimum of CO <sub>2</sub> +O <sub>2</sub>	At total absorption of oxygen	At onset of black heart
C.	Days	Days	%	%	%	%	Days	Days	%	%	Days	CO <sub>2</sub> O <sub>2</sub>	CO <sub>2</sub> O <sub>2</sub>	CO <sub>2</sub> O <sub>2</sub>
0	3 to 8	37	22.2	0	0.60	0.7	30	7	16.6	4.4	23	0.79	0.86	1.06
2.5	5 to 8	47	22.8	0	0.49	0.6	36	11	14.6	6.4	21	0.60	.90	1.08
5	7	70	25.0	0	0.36	0.7	28	42	16.6	4.4	21	0.73	.80	1.14
7.5	4 to 7	50	19.0	0	0.38	0.6	35	15	13.6	7.4	16	0.49	.73	0.91
10	5 to 9	42	25.6	0	0.6	1.0	21	21	17.2	3.8	14	0.80	.99	1.22
15	4	34	30.8	0	0.9	1.0	22	12	16.4	4.6	10	0.70	.86	1.47
			(24.7)*		(0.7)*									(1.18)*
20	2	12	23.4	0	2.00	2.1	10	2	17.4	3.6	6	0.81	.97	1.11
25	1	7	20.4	0	2.9	3.0	7	0	18.6	2.4	5	0.84	.97	0.97
30	1	5	20.0	0	4.0	4.2	5	0	18.6	2.2	3	0.86	.95	0.95
35	1	3	15.8	3.0	5.3	4.2	5		18.0	3.0	2	0.81	1.21	0.88
40	1/24	7/24	5.0	15.2	17.1	19.9			19.6	1.4	9/24	0.81	1.19	0.86
45	1/24	4/24	3.6	16.6	21.6	26.4			19.8	1.2	8/24	0.81	0.81	0.82

\* The values given at these points are exceptionally high. In parentheses are given values based upon the next preceding and succeeding jars, which are much more nearly representative of the series at this point.



TABLE 4

THE RATE OF PRODUCTION OF CARBON DIOXIDE BEFORE AND AFTER THE EXHAUSTION OF FREE OXYGEN FROM THE AIR IN THE CONTAINERS

Temperature C.....	20	15	10	7.5	5	2.5	0
Average daily production of carbon dioxide before exhaustion of free oxygen. %	2.62	1.10	1.03	0.48	0.59	0.48	0.58
Average daily production of carbon dioxide after exhaustion of free oxygen and before the appearance of blackheart. %	0.92	0.78	0.19 (0.09) (0.34) (0.12) (0.32)	0.27	0.25	0.35 (0.68) (0.35) (0.13)	0.43 (0.28) (0.40) (0.60)

In table 5 are shown the effects of storage for several weeks at 0° C. and 5° C. on the subsequent production of blackheart in Netted Gem tubers. After being taken out of storage, tubers from each lot were placed in an oven, having access to normal air at 40° C. One lot from each series was tested at the stated intervals in order to note the progressive development of blackheart in the two series.

TABLE 5

COMPARATIVE AMOUNTS AND SEVERITY OF BLACKHEART DEVELOPED IN NETTED GEM TUBERS, PREVIOUSLY STORED AT 0° C. AND 5° C.\*

Storage temperature C.	Hours heated at 40° C.					
	21	30	42	66	90	114
0	2+	2+	3+	2+	1+	1+
	3-	3-	2-	2++ 1+++	2+ 2+++	4+++
5	1+	1+	2+	2+	1+	2+
	4-	4-	3-	3-	1++ 3-	2+++ 1-

\* Number of tubers examined and presence and severity of blackheart indicated as in previous tables.

In table 6 are shown the effects of previous storage of Netted Gem tubers at 0° C. and at 5° C., on the subsequent gaseous exchange and production of blackheart when the tubers were placed in sealed chambers at 40° C., with a content of three volumes of air to one volume of tubers.

TABLE 6

THE EFFECT OF PREVIOUS STORAGE OF TUBERS AT 0° C. AND AT 5° C., ON THE GASEOUS EXCHANGE AND PRODUCTION OF BLACKHEART IN SEALED CHAMBERS AT 40° C.

	Time hours	2	3	4	5	6	7	8	9
Stored previously at 0° C.	CO <sub>2</sub> evolved %	2.0	3.6	5.2	8.0	6.8	11.2	12.2	.....
	O <sub>2</sub> remaining %	19.8	17.4	16.6	15.8	16.6	13.2	13.4	.....
	CO <sub>2</sub> +O <sub>2</sub> present % .....	21.8	21.0	21.8	23.4	23.4	24.4	25.6	.....
	Tubers and blackheart.	8—	4— 2+ 1++	4+ 3++	4+ 2++ 2+++	3+ 3++ 2+++	3— 3+ 2++	1— 3+ 3++ 1+++	
Stored previously at 5° C.	CO <sub>2</sub> evolved %	.....	.....	2.8	3.4	6.0	4.8	.....	6.0
	O <sub>2</sub> remaining %	.....	.....	17.2	17.8	14.6	16.2	.....	15.4
	CO <sub>2</sub> +O <sub>2</sub> present % .....	.....	.....	20.0	21.2	20.6	21.0	.....	21.4
	Tubers and blackheart.	.....	.....	8—	5— 5+	3— 5+	1— 4+ 4++	.....	2+ 4++ 1+++

## DISCUSSION

### 1. THE BEHAVIOR OF DIFFERENT VARIETIES

At the highest temperatures no great difference appeared in the behavior of the three varieties of tubers used. In the experiments at 35° and 40° C., blackheart appeared at approximately the same time in each case. At these high temperatures the carbon dioxide evolved was relatively low and the oxygen residue in the jars relatively high in amount at the time when injury occurred. At 30°, 25° and 20° C., marked divergence in behavior appeared. The Rural New Yorker and Green Mountain Jr. tubers were injured two or three days earlier than those of the Netted Gem variety. At these lower temperatures the

carbon dioxide production was relatively large and the oxygen residue relatively low at the time of injury. At 35° and 40° C., blackheart can be produced by heating tubers in normal air; at 30° C. this occurs infrequently and at 25° C. very rarely. Under the conditions of accelerated respiration in the heated tubers a slight reduction in the oxygen concentration of the surrounding atmosphere decreased the rate of entrance of oxygen sufficiently to bring about its more rapid depletion in the tissues so that very early injury resulted. Owing to the rapidity of respiration at these higher temperatures differences in tubers of different varieties were largely obscured. At the lower temperatures of 20° to 30° C., the lower rate of respiration allowed the oxygen supply within the tissues to be maintained sufficiently high to delay injury until most of the oxygen had been removed from the atmosphere of the jars.

At 35° C., the consumption of oxygen in respiration appeared to be very nearly balanced with the rate of oxygen entrance. Injury produced by heating in normal air occurred only after several days exposure to this temperature and never appeared in all the tubers as at 40° C. A small reduction of oxygen concentration within the jars at 35° C. sufficed to retard oxygen entrance sufficiently to bring about deficiency of oxygen within the tissues, resulting in early injury. At 40° C. the rate of oxygen entrance was far from being equal to its rate of use, so that injury resulted still more quickly to tubers within the jars. At 30° C. heating in normal air infrequently results in injury and then only in a very few tubers and after long exposure. When the tubers were enclosed in jars at this temperature a much greater reduction of oxygen concentration, with correspondingly high concentration of carbon dioxide, occurred before depletion of oxygen in the tissues was sufficient to cause injury. This is still more evident in the experiments at 25° and 20° C. At the latter temperatures, Rural New Yorker and Green Mountain Jr. tubers reduced the oxygen concentration to 2.8 per cent and 1.0 per cent, respectively, before injury occurred, while Netted Gem tubers reduced it to zero.

The differences in the behavior of the different varieties are probably due in part to differences in permeability of the skin of the tubers to oxygen. Bartholomew found that removing the skin prevented or greatly delayed injury in tubers heated to 40° C. He also found<sup>3</sup> that surrounding the tubers with a high concentration of oxygen dur-

ing the period of heating prevented the injury. Both these results point to the skin as a highly important factor in the susceptibility of tubers to the development of blackheart. It is significant that Appleman<sup>1</sup> found the degree of suberization of the skin to have a marked effect upon the rate of respiration of potatoes. The existence of these marked differences in the permeability of the skins of tubers of different varieties does not appear to be generally recognized. Whether or not there are inherent differences in the activity of the protoplasm of different varieties appears to be unknown. There may be such differences, but it seems improbable that they had as much influence on the results of the present experiments as the permeability of the skin, since there was little difference in the behavior of the three varieties when inclosed in jars at high temperatures. The two factors, if both were present, cannot be distinguished with the available data.

## 2. THE EXCHANGE OF GASES

It has been pointed out that at the higher temperatures the carbon dioxide content of the jars was relatively low and the oxygen content relatively high at the time blackheart appeared. This applies to the series at 45° and 40° C. for all varieties used and to Rural New Yorker and Green Mountain Jr. tubers at 35° C. At 35° C. the jars containing Netted Gem tubers showed a relatively high carbon dioxide and low oxygen concentration when blackheart appeared. At temperatures of 30° to 20° C., the carbon dioxide content of the jars was always relatively high and the oxygen content relatively low when blackheart occurred. In the series of jars at 30° to 20° C. containing Rural New Yorker and Green Mountain Jr. tubers, blackheart always occurred while a measurable amount of oxygen was still present in the jars, but where Netted Gem tubers were used blackheart did not appear in any case until the oxygen had been completely removed from the atmosphere of the jars. This brings out a marked difference in the behavior of the different varieties used. Bartholomew found that Rural New Yorker and Green Mountain Jr. tubers were readily injured by an exposure of 15 to 24 hours in normal air at 40° C. Netted Gem tubers, however, required an exposure of 24 to 48 hours under these conditions before injury occurred. This greater difficulty in producing injury in Netted Gem tubers was reflected in the results obtained when tubers were confined in jars. The lesser susceptibility of the Netted



Gem tubers allowed a greater accumulation of carbon dioxide and depletion of oxygen before the first appearance of blackheart in the series. This difference may have been due in a large measure to the greater permeability of the skins of Netted Gem tubers to oxygen.

As the temperature was lowered still further in the experiments with Netted Gem tubers a striking result appeared. At 30° C. and 25° C. injury appeared on the same day that the oxygen content of the jars fell to zero. At 20° C. injury was delayed until two days after complete removal of oxygen from the atmosphere of the jar. As the temperature was lowered still further this period of delay increased until at 5° C. the tubers were exposed to an atmosphere entirely devoid of oxygen for 42 days before injury appeared. With decrease of temperature below 5° C., the period without free oxygen necessary to produce injury decreased rapidly to 7 days at 0° C. This is shown in table 3, column 3. It seems remarkable that at any temperature living tissues of the type of potato tubers could withstand exposure to an oxygen-free atmosphere for 42 days before injury occurred. In all the series at different temperatures the tubers were in every way apparently normal and uninjured in every jar opened previous to the time of appearance of blackheart.

Some light may be thrown on these results by an examination of the data which show the absorption of oxygen and production of carbon dioxide (table 3, columns 4 and 5). It is evident from these values, in the range of temperature where oxygen was entirely removed from the atmosphere of the jars before blackheart occurred, that the carbon dioxide produced was in nearly all cases equivalent to or slightly greater than the amount of oxygen originally present in the atmospheres of the jars, i.e., 21 per cent by volume. The oxygen rapidly absorbed in the early part of the experiment evidently served for the continuance of normal respiration after the oxygen had been completely removed from the surrounding atmosphere.

This table also brings out the fact that at all the temperatures, and especially at the lower temperatures, oxygen was absorbed much faster than carbon dioxide was produced. This difference caused a decrease in the sum of the oxygen and carbon dioxide present in the jars, the maximum decrease occurring later at the lower temperatures. Where the difference of rates was marked, a depression of the gas pressure occurred in the jars which in some cases was equivalent to a few centimeters of mercury.

It has long been known that the respiratory ratio frequently falls far below unity in plant tissues. Bonnier and Mangin<sup>5</sup> showed that with germination of starchy and proteinaceous seeds it was approximately unity for the first stages of germination and then fell below this value with increasing growth rates. Palladin<sup>12</sup> showed that rapidly growing internodes of various plants absorbed oxygen more rapidly than they produced carbon dioxide. The same investigators and others have shown that during the germination of fatty seeds the respiratory ratio is less than unity at first, gradually rising to near unity later. In all these cases very active tissues were used and the low value of the respiratory ratio was ascribed to the utilization of oxygen in the formation of substances requiring the addition of oxygen during synthesis. In mature, dormant or resting tissues the respiratory ratio at ordinary temperatures has commonly been found to be near unity except in the succulent plants which are noted for their accumulation of organic acids as a result of respiration (Puriewitsch;<sup>13</sup> Richards<sup>14</sup>). The minimum respiratory ratio was usually found to occur at 10° to 15° C. In the present experiments, before definite injury to the tubers occurred, the respiratory ratio was below unity though the whole range of temperature, and the greatest decrease from unity was found in the lower half of this range. Column 13, table 3, shows the values of  $\text{CO}_2/\text{O}_2$  calculated from the results for the jars showing the lowest sum of carbon dioxide and oxygen in each series. The lowest value, 0.49, shows that during the first five weeks of the series at 7½° C., oxygen was absorbed on an average twice as rapidly as carbon dioxide was produced. Since this same phenomenon occurred in the early part of each series, it appears to be a normal occurrence with potato tubers and not a result of confinement in the jars. This point has not been studied under other experimental conditions.

In the light of the above data it appears probable that any supply of oxygen less than that necessary to maintain free oxygen in the atmosphere surrounding the tubers for one-half to all of their storage period in the range below ordinary room temperature, would be liable to result in blackheart. The amount of oxygen necessary to prevent the injury cannot be stated definitely but depends upon the temperature, the length of the storage period and the state of activity of the tubers, this depending upon the variety and the previous treatment. It appears highly improbable that such low oxygen concentration as is



required for the production of blackheart at low temperatures could ever develop in commercial cold-storage plants. It might possibly develop in unventilated storage pits. At higher temperatures it is obviously necessary to maintain a supply of air around the tubers sufficient to prevent the concentration of oxygen falling appreciably below that of normal air.

What the conditions may be which lead to the production of blackheart while the tubers are yet in the soil, are little known. Comparisons between tubers planted in jars of soil and tubers surrounded only by normal air, showed no significant differences. Temperature appeared to be the predominant factor. In the field, however, the conditions of aeration are probably quite different than in jars of soil, and the composition of the soil atmosphere may play a part. The high oxygen content known generally to exist in the upper layers of soil under good cultural conditions, indicates that temperature is the predominant factor. However, the high rate of oxygen absorption and carbon dioxide production by tubers at elevated temperatures, coupled with the limited volume of air in the soil and its slow diffusion through the soil, may quite possibly lead to a much different composition of the soil atmosphere in the region occupied by a hill of tubers, than in unoccupied regions. In addition, a high water content of the soil may be an important factor due to its relation to the air content. Some of the cases of blackheart reported from the field have been associated with early autumn rains coincident with moderately elevated temperatures.

### 3. THE RATE OF RESPIRATION AT DIFFERENT TEMPERATURES

In all cases a lowering of temperature in the range between 45° and 5° C., caused a decrease in the rate of respiration as indicated by carbon dioxide production. A lowering of 10° C. roughly halved the respiration rate, in agreement with the Van't Hoff-Arrhenius rule. There was large variation between the lots in different jars of the same series. This was probably due to variation among individual tubers and the use of a relatively small number of tubers in each jar. In producing blackheart by heating tubers to 38° to 40° C., it was found by Bartholomew that great variation existed in the response of individual tubers. Some showed severe, some slight and some no injury, when treated in exactly the same manner. While no evidence of varia-

tion in individual tubers was obtained by measurement of carbon dioxide production, it seems certain, from Bartholomew's observations and the results given in the present tables that such variation existed. It is unknown whether or not this variation was due to differences in the permeability of the skin to oxygen or to differences in the activity of the protoplasm.

In the range of  $5^{\circ}$  to  $0^{\circ}$  C., the effect of lowering the temperature was the reverse of that at higher temperatures. Tubers in jars at  $0^{\circ}$  C., were injured by one-half the period of exposure required to produce corresponding injury at  $5^{\circ}$  C. The actual periods were 37 days and 70 days for  $0^{\circ}$  and  $5^{\circ}$  C., respectively. At  $2\frac{1}{2}^{\circ}$  C., an exposure of 47 days was required to produce injury, and at  $7\frac{1}{2}^{\circ}$  C., an exposure of 50 days. The rate of respiration at the different temperatures was inversely proportional to the period required to produce injury. The respiration rate was markedly higher at  $0^{\circ}$  C. and at  $2\frac{1}{2}^{\circ}$  C., than at  $5^{\circ}$  C., and increased with temperature above  $5^{\circ}$  C.

There is indication in table 2, that the rate of respiration at  $0^{\circ}$  C. undergoes a marked change during the earlier part of the period of exposure. During the first week the carbon dioxide production was markedly lower at  $0^{\circ}$  than at  $5^{\circ}$  C. By the end of the first two weeks this difference had disappeared, and at the end of three weeks the tubers at  $0^{\circ}$  C., showed a larger total production of carbon dioxide than those at  $5^{\circ}$  C. The tubers used in the  $0^{\circ}$  C. series were stored at  $5^{\circ}$  C., previous to use in the experiment. When placed in the jars at  $0^{\circ}$  C., their immediate response was a slowing of the respiration rate, succeeded by a gradual acceleration of this rate until it exceeded that of tubers at  $5^{\circ}$  C. In two parallel series of jars at  $0^{\circ}$  C., containing tubers of the same variety and lot but with one containing tubers stored at  $5^{\circ}$  C., and the other at  $0^{\circ}$  C., for several weeks previous to use, the carbon dioxide production in the latter series was higher from the beginning than in the former. This is shown in table 4.

The observation that tubers show an increased rate of respiration at temperatures near  $0^{\circ}$  C., seems to have first been made by Müller-Thurgau.<sup>10, 11</sup> He held this to be due to the increased sugar content which occurs in potato tubers under such conditions of storage. The greatest accumulation of sugar was found by him to occur at  $0^{\circ}$  C. At higher temperatures the accumulation was less, the sugar content becoming normal at  $6^{\circ}$  to  $10^{\circ}$  C. The accumulation of sugar at  $0^{\circ}$  C.

was found to be gradual, reaching a maximum of 2 to 3.5 per cent of the fresh weight in two or three months. Different tubers showed great variation in the amount of sugars accumulated during equal periods, and in the maximum amount formed. At temperatures of 10° C., or higher, the normal content of sugar varied from none in new tubers to a maximum of about one-half of one per cent in old tubers.

The findings of Müller-Thurgau were confirmed by Boehm<sup>4</sup> and Butler,<sup>6</sup> and similar observations were made by Appleman.<sup>2</sup> These investigators appear to have carried out most of their measurements of respiration at room temperature. Müller-Thurgau, however, gives results for a comparison of sweet and non-sweet tubers at 0°, 10°, 20° and 25° C. At all these temperatures the sweet tubers, made so by previous storage at 0° C., showed a much higher rate of respiration than non-sweet tubers. Of most significance in relation to the present study, is the fact that in his experiments sweet tubers produced carbon dioxide about 40 per cent faster at 0° C., than did non-sweet tubers at 10° C. In the present experiments the respiration rate at 0° C. did not equal that at 10° C. The tubers used at 10° C., however, had been stored previous to use, at 5° C., at which temperature, according to Müller-Thurgau, some accumulation of sugar occurs. No determinations of sugar were made in the present study. However, the tubers in the jars at 0° C., removed after the second week, had a decidedly sweet taste.

None of the experiments of the investigators mentioned were carried out within the range of temperature between 0° and 10° C., and in all of them the respiration rate was measured over a relatively short period of a few hours to a few days. Ziegenbein's<sup>16</sup> measurements of the effect of temperature upon the rate of respiration of potato tubers were not carried below 10° C., except in one experiment at 0° C. for a very short period. The results of all these previous studies agree with those presented here for the corresponding temperature range.

The effect of exhaustion of free oxygen from the jars on the rate of carbon dioxide production is shown in table 4. In most cases this rate was reduced to one-half to two-thirds of its previous value with free oxygen present. The accumulated oxygen was apparently not as readily usable as free oxygen but sufficed to prevent the appearance of injury for an extended period at the lower temperatures. In this

table also, it appears that the respiration rate decreased till within the range of  $5^{\circ}$  to  $7\frac{1}{2}^{\circ}$  C., then showed no further decrease with decrease of temperature, but a distinct increase even with free oxygen absent. The values given are averages of the rates of all the jars at a given temperature removed during the two periods under comparison. To what extent, if any, the accumulation of carbon dioxide contributed toward the decrease in rate of respiration is unknown. The greatest change in rate occurred at about the time of exhaustion of free oxygen so that this appears to have had more effect than the gradual accumulation of carbon dioxide in the jars.

The existence of a minimum rate of respiration of potato tubers when stored at low temperatures for long periods, at approximately  $5^{\circ}$  C., does not appear to have been known previously. Measurements of respiration made over short periods, and when the tubers had very recently been placed at low temperatures would not show this. The existence of the minimum rate at  $5^{\circ}$  C., seems probably due to the occurrence at this point of an equilibrium between the opposing effects upon the respiration process of decreased temperature, and increased sugar content or factors correlated with it. This equilibrium point may vary somewhat with different varieties, and at different times in the life of the tubers. It probably varies also for different individual tubers of the same variety, for Müller-Thurgau found that the capacity to accumulate sugar varied with different tubers. Whether or not such a minimum point occurs in the respiration of other vegetable tissues is unknown. Müller-Thurgau<sup>10</sup> found that grape leaves, stalks of kohlrabi, and germinating hempseeds accumulated sugar when stored near  $0^{\circ}$  C. But no study appears to have been made of the respiration of these tissues.

#### 4. INTERNAL CONDITIONS AND DISTRIBUTION OF INJURY IN TUBERS

In what form the excess of absorbed oxygen is held within the tuber is not known. Müller-Thurgau found an increase in the content of free malic acid in tubers placed at low temperatures, presumably the result of incomplete oxidation of certain substances within the tuber. This may, at least in part, account for the excess of oxygen absorbed by the tubers, as in the case of succulent plants. It seems possible also that the excess oxygen may become attached to some autoxidizable substance in the tuber as a peroxide which accumulates during the early



part of the life of the tuber and is utilized in the more rapid respiration occurring in later stages, when stored under normal conditions. In whatever form it occurred, it apparently was utilized by the tubers in the present experiments, since they showed a steady production of carbon dioxide without injury, although at a slower rate, for a considerable period after the exhaustion of free oxygen.

When the accumulated oxygen in the tubers disappeared, injury soon occurred. In table 3, column 15, is shown the carbon dioxide-oxygen ratio for the periods up to the time when blackheart appeared. In all cases, except one, it is close to or above unity. This indicates that as the tubers confined in a jar continued to respire, the free oxygen was absorbed and used until exhausted, then the oxygen accumulated in the tissues was utilized and the tissues maintained in apparently uninjured condition until the approximate equivalent of the oxygen originally available to the tubers had been converted into carbon dioxide. After this point had been reached the tissues were forced to respire anaerobically with injury rapidly resulting.

When blackheart was produced in tubers at higher temperatures, 20° C. and upward, whether in normal air or confined in jars, the injury was found to be entirely internal (plate 1), usually centrally located in the tubers. At temperatures below 20° C., in tubers confined in jars, the injury was usually both internal and on the surface of the tubers (plate 2). Surface injury often extended over one-half of the surface and into the tissues for several millimeters in severe cases, especially at the lower temperatures. Frequently, also, the injury appeared in very numerous small spots throughout the tuber (plate 3) giving an appearance resembling "frost necrosis" reported by Jones,<sup>7</sup> but not predominating in the region of the vascular ring. In such tubers the typical large irregular injured areas usually appeared as well as the surface injury. Surface injury was noted by Stewart and Mix in their experiments. The other type of injury, resembling "frost necrosis," was not mentioned by them, and may not have appeared in their experiments.

In the experiments reported here, surface injury never occurred, except in jars in which all the free oxygen had been exhausted, for sometime before its appearance. Under these conditions the accumulated oxygen apparently became well distributed and was used up about equally in all parts of the tuber. Injury then occurred at any

point in the tuber where oxygen deficiency occurred. At the higher temperatures, however, the appearance of injury coincident with or before the exhaustion of free oxygen indicated that uniform distribution of either free or accumulated oxygen did not occur, and injury resulted rapidly in those tissues farthest removed from the source of supply. It is possible also that the accumulated oxygen in the tissues is not normally equally distributed throughout the tuber, but is largely held in the outer tissues, making these much less liable to injury by oxygen depletion. Oxidase action was found to be most marked in the outer tissues as indicated by the direct blueing of guaiacum tincture, which is dependent upon the presence in the tissues of a substance equivalent to hydrogen peroxide in its relation to the reaction involved. The tissues immediately outside the vascular ring were always the last to succumb at low temperatures.

The presence of surface injury when tubers were injured at low temperatures through exhaustion of oxygen was so generally present as to constitute an indication of the conditions under which injury had occurred. On the other hand, the appearance of internal injury alone in large areas indicated that the injury had occurred at high temperatures with little or no depletion of oxygen in the atmosphere surrounding the tubers. This is in complete agreement with the statements of Stewart and Mix.<sup>15</sup>

The accumulation of carbon dioxide in the jars appeared to have no relation to the injury. When excessively high concentrations of carbon dioxide occurred, in some cases 30 per cent or over, the tubers usually were flabby and exuded water, resembling tubers which had been frozen and thawed. Blackheart was always severe in such cases but had usually appeared earlier in the series. It is presumed that the carbon dioxide concentration within the tissues was always as high as, and probably higher at all times, than in the surrounding atmosphere. This was found to be the case by Magness<sup>8</sup> in his study of the composition of the gases in the intercellular spaces of apples and potatoes. With both of these, increase of temperature increased the proportion of carbon dioxide to oxygen in the tissues. The low amount of oxygen found by Magness in the tissues exposed at the higher temperatures lends support to the belief that anaerobic conditions develop within the tissues before blackheart occurs.



The accumulation of sugar in tubers, induced by long exposure to low temperatures, apparently made them somewhat more susceptible to injury when heated at 40° C. in normal air. Tables 5 and 6 show the results of such experiments. The increased rate of respiration found associated with increased sugar content by Müller-Thurgau, and shown to occur at higher as well as lower temperatures, would be expected to hasten the approach of conditions in the tuber which would result in its injury. This appeared to be the case, since for equal periods of exposure to 40° C., in normal air, more tubers were injured in the early stages in the lot previously held at 0° C., than in that held at 5° C. In the later stages injury was also much more severe in the former lot. When lots of the same tubers were sealed in jars the same results appeared and the gaseous exchange agreed with that previously described for tubers stored in sealed jars for long periods at 0° and 5° C. It may be safely stated that tubers which have been subjected to low temperatures sufficiently long to cause them to accumulate sugar in their tissues are more liable to develop blackheart than normal tubers when subjected either to high temperature, or to low temperature and depleted oxygen supply.

### SUMMARY

1. A definite relation between temperature, oxygen supply, and period of exposure required to produce blackheart, has been shown.

2. Differences in the readiness with which the different varieties used could be injured by heating at high temperatures in normal air were largely eliminated when the tubers were heated while confined in jars with six volumes of air to one volume of tubers.

3. When tubers were confined in jars and heated to temperatures of 20° to 30° C., which are too low to cause injury with the tubers surrounded by normal air, differences in behavior appeared which corresponded to the differences exhibited by the different varieties when heated in normal air at 35° to 40° C. Netted Gem tubers at 20° to 30° C. required a longer period of heating than the other two varieties to produce injury, and this did not occur until the free oxygen content of the jars had been entirely exhausted. Rural New Yorker and Green Mountain Jr. tubers were injured more quickly and always before the disappearance of the free oxygen in the jar.

4. Differences in the behavior of the different varieties at high temperatures may be due to differences in the permeability of the skins to oxygen. All the evidence points toward the permeability of the skin as an important factor affecting the rate of respiration of potato tubers.

5. As the temperature was lowered, longer periods of confinement in the jars were necessary to produce injury, until  $5^{\circ}\text{C}$ . was passed. At temperatures of  $2.5^{\circ}\text{C}$ . and  $0^{\circ}\text{C}$ ., injury occurred more quickly than at  $5^{\circ}\text{C}$ .

6. Lowering the temperature caused a decrease in the rate of respiration as indicated by the carbon dioxide output, in accordance with the Van't Hoff-Arrhenius rule, between  $45^{\circ}\text{C}$ ., and  $5^{\circ}\text{C}$ . Decreasing the temperature below  $5^{\circ}\text{C}$ . caused an increase in the rate of respiration. The rate of respiration at  $0^{\circ}\text{C}$ . was nearly as high as at  $10^{\circ}\text{C}$ . The existence of a minimum rate of respiration at  $5^{\circ}\text{C}$ . appears to be the result of an equilibrium between the decreasing effect of a lower temperature, and the increasing effect of an increased sugar content on the respiration process as shown by other investigations.

7. In all cases, oxygen was absorbed more rapidly than carbon dioxide was produced during the early part of the experiments, and the respiratory ratio at the lower temperatures was less than at the higher temperatures.

8. At low temperatures injury to tubers did not occur until a considerable period had elapsed after the exhaustion of the oxygen from the atmosphere of the jar. The maximum period of exposure to an atmosphere devoid of oxygen before injury occurred was 42 days at  $5^{\circ}\text{C}$ .

9. Injury did not occur at the lower temperatures until the approximate equivalent of the available free oxygen had been returned as carbon dioxide to the atmosphere of the jar.

10. The rate of respiration before exhaustion of free oxygen was about twice as great as afterwards. The tubers apparently used the oxygen accumulated in the tissues, but with more difficulty than the free oxygen from the surrounding atmosphere.

11. The actual injury evidently resulted when anaerobic conditions were brought about in the tissues, and may be attributed to processes initiated as a result of those conditions.

12. Tubers confined at 20° C., or higher, invariably showed only typical internal blackheart as described originally by Bartholomew. Tubers injured at 15° C., or below, usually showed both internal and surface injury, and occasionally a diffuse injury throughout the tissues.

13. The accumulation of carbon dioxide in the jars appeared to have no close relation to the production of blackheart.

14. Tubers exposed to low temperatures until they became sweet appeared to be more easily injured by high temperatures than non-sweet tubers.

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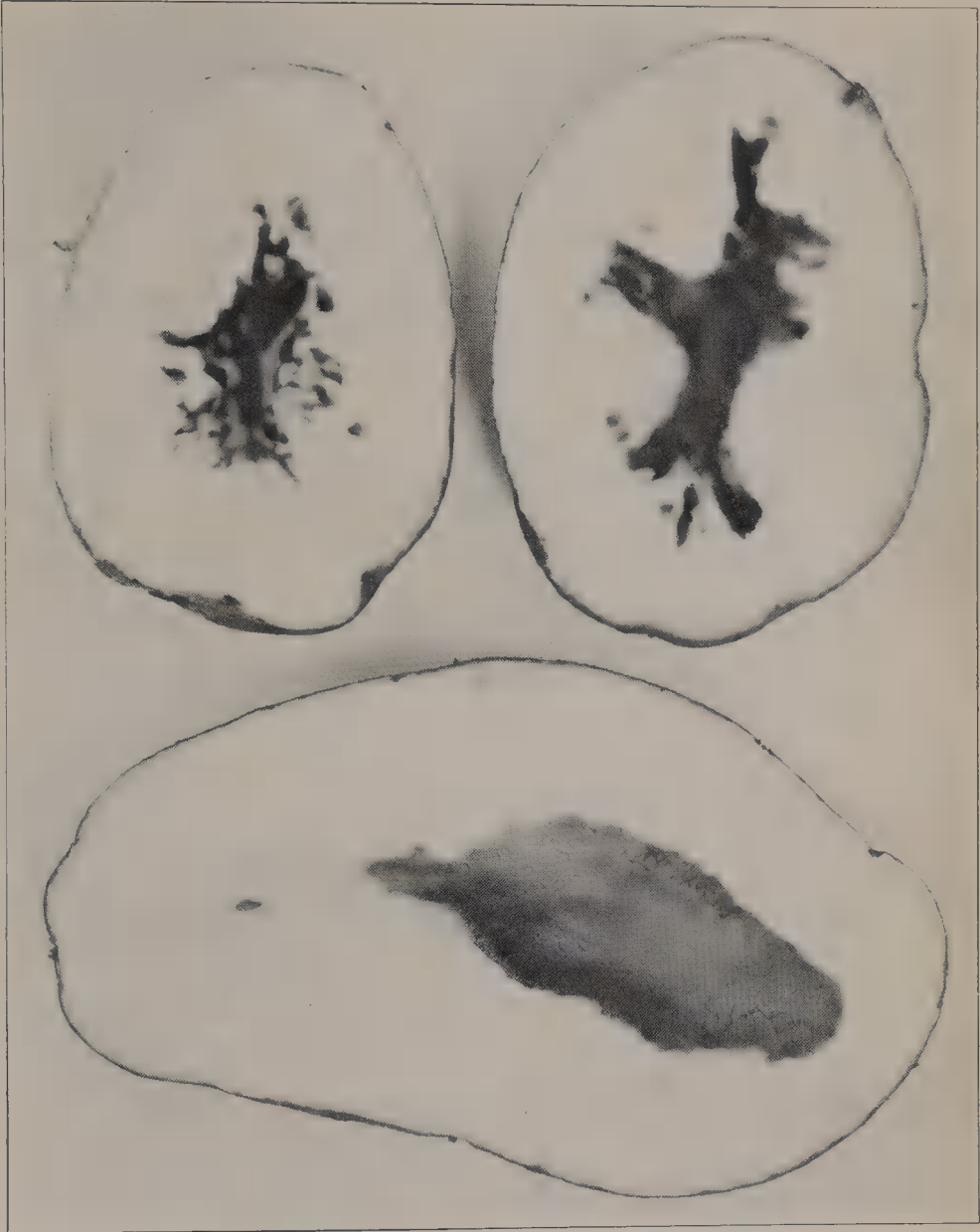
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PLATE 1

Typical blackheart produced by high temperature. No injury appears on the surface of the tubers.









## PLATE 2

Severe blackheart produced at low temperature ( $2\frac{1}{2}^{\circ}$  C.) with deficiency of oxygen in sealed container. Severe injury appears in interior and on surface of tubers.

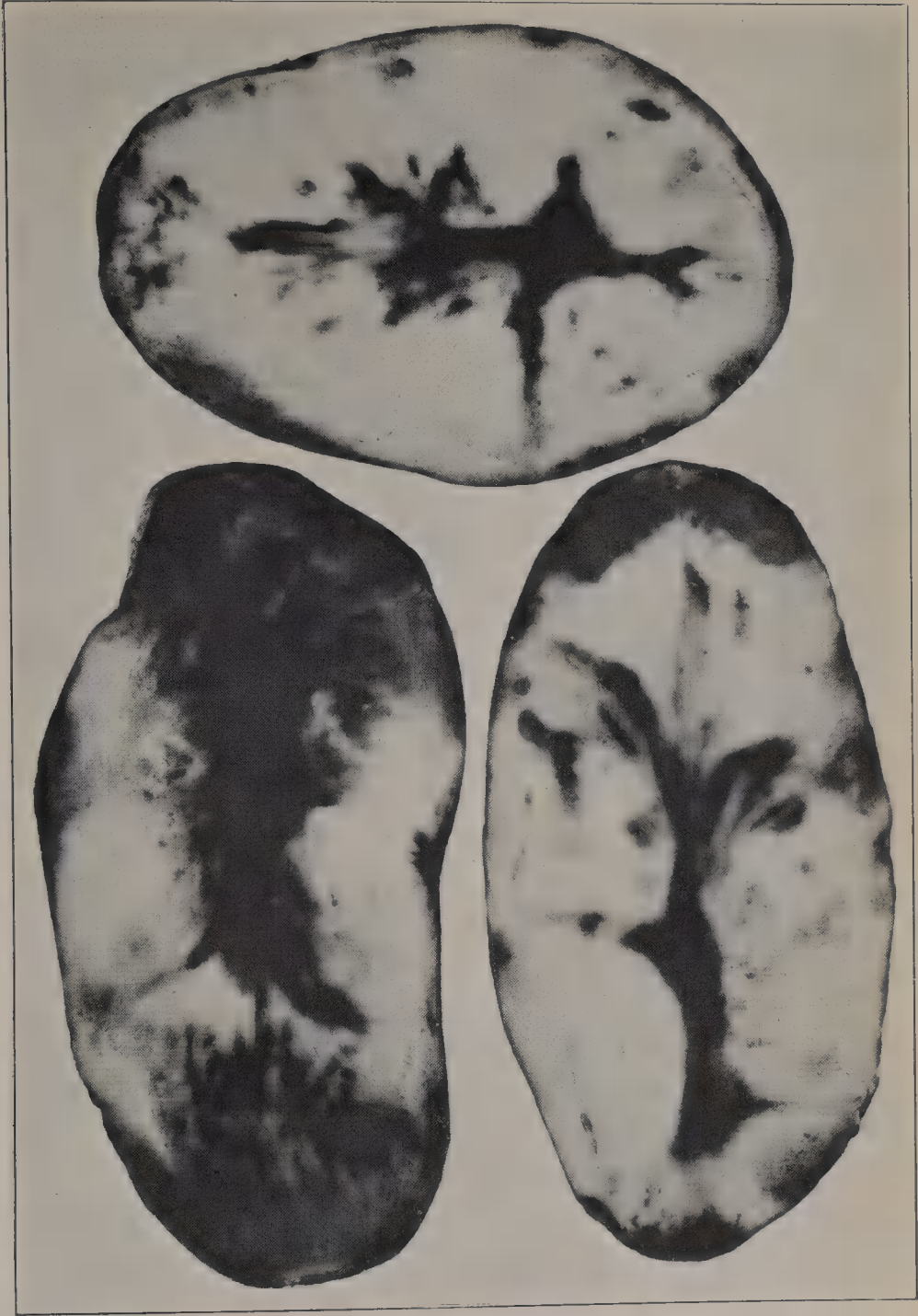


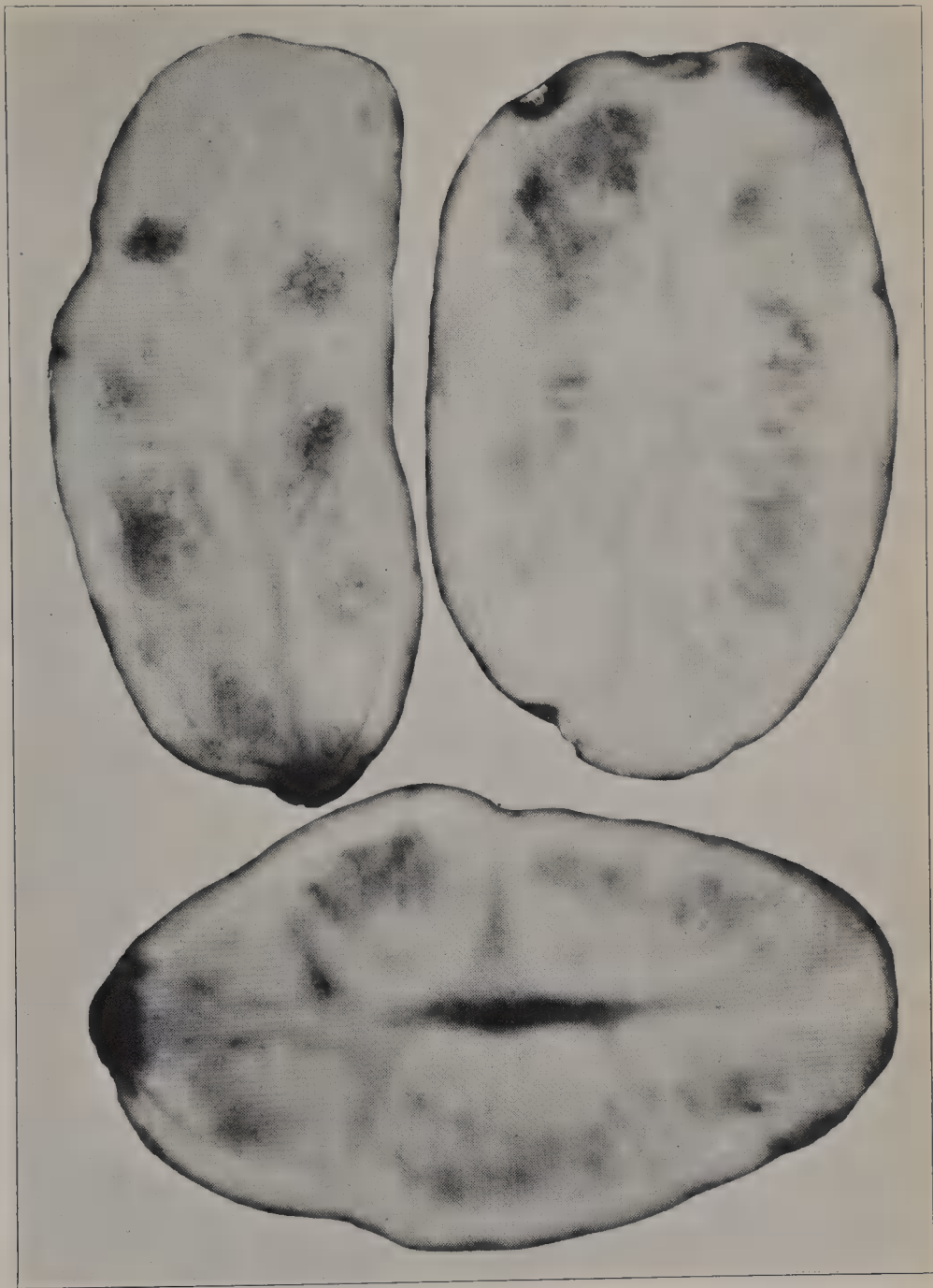






PLATE 3

Diffuse type of injury occurring occasionally in tubers kept in sealed containers at low temperatures.





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## REPLACEABLE BASES IN SOILS\*

BY

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### INTRODUCTION

Former papers from this laboratory<sup>7, 22, 23</sup> have set forth the results of studies on the effects of salts on soils. In harmony with many previous workers we have shown that the reactions which take place when neutral salt solutions are used are of the nature of an exchange of bases; a part of the base of the solution is absorbed by the soil and some one or more of the bases of the soil pass into the solution. The exchange of bases is stoichiometric, or approximately so. The bases thus brought into solution are referred to as being replaced. With neutral or alkaline soils these bases are mainly calcium, magnesium, potassium and sodium, but as will be shown later acid soils may contain still other replaceable cations.

Two hypotheses, one chemical and the other physical, have been advanced to account for these reactions. The first was proposed by Way<sup>41</sup> in 1852 as a result of his studies on certain artificially prepared aluminosilicates. Twenty-five years later van Bemmelen<sup>37</sup> accepted the essentials of Way's views and adopted the suggestion previously made by others that the compounds involved in the replacement reactions are of a zeolitic nature. As is well known van Bemmelen<sup>33</sup> abandoned this view about ten years later as a result of his studies on colloidal materials. He then concluded that the replaceable bases are not chemically combined with the soil but are present in a state of adsorption. This latter view has since been quite widely accepted, although not universally so.

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\* Paper No. 119, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

The fact that soil has the power of absorbing potassium and ammonium, a power which enables it to retain these constituents of fertilizers against the leaching action of rains, has attracted the attention of soil workers for three-quarters of a century.\* Largely because of the dominance of the so-called plant-food doctrine, comparatively little study has been devoted until recently to the relationships between the replaceable bases and the general chemical and physical properties of the soil mass. Soon after taking up a study of this subject about six years ago the senior author became convinced that the effects produced by soluble salts as a result of replacement reactions may have far-reaching consequences. Recent publications from this and other laboratories<sup>7, 8, 11, 20, 23, 31</sup> have abundantly confirmed this view. We may assert with confidence that significant changes take place in certain of the solid components of soils as a result of the substitution of one base for another.

It now seems certain that some of the most difficult phases of the alkali problem of semi-arid regions are closely related to and indeed caused by the substitution of sodium for one or more of the bases normally present in replaceable form. There are two especially important effects produced by the substitution of sodium for the divalent bases, namely: (1) the granular structure of the clay materials becomes broken down with the resulting development of extreme impermeability; (2) sodium carbonate is formed as a result of hydrolysis. The second of these effects has been discussed by Cummins and Kelley<sup>7</sup> and others;<sup>8, 11, 31</sup> the first will be the subject of a later communication (see 12).

A moment's reflection suggests that those alkali soils which contain the greatest amount of replaceable bases are likely to contain the greatest amount of absorbed sodium. Our studies have shown this to be true. We now know that in the reclamation of alkali soils the absorbed sodium may necessitate the application of special treatments. While this fact has been emphasized by several workers in this field, it is doubtful whether many students of alkali soils fully appreciate its significance.

The replaceable bases of soils which do not contain high concentrations of soluble salts also perform important functions which have

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\* It seems unnecessary to give an extended bibliography of this subject. Those who are interested in its historical development should consult papers by Patten and Waggaman,<sup>29</sup> Sullivan,<sup>34</sup> Prescott<sup>30</sup> and Fisher.<sup>9</sup> The more important recent publications are cited below.

received scant attention. The constituents involved in basic exchange are among the most labile and reactive components of soils. Nevertheless, current literature and standard texts make but brief reference to the relationship between the replaceable bases and such questions as the soil solution, soil acidity, the physical properties of soils, etc.

The vast majority of the former students of this subject have not determined the total content of the several replaceable bases nor their relative proportions. Most of the previous investigations have been made by bringing a salt solution to approximate equilibrium with the soil. Usually it is not possible to effect complete replacement in this way. A few investigators have subjected samples of soil to the leaching action of salt solutions. It is only comparatively recently, however, that serious effort has been made to determine the total content of the bases present in replaceable form, their relative proportions and their effects on the chemical and physical properties of the soil. The result has been that some very important relationships have passed largely unobserved.

As is well known the inference has been drawn that the capacity of soils to absorb potassium and ammonium bears some ill-defined relation to the clay content. Anderson *et al.*<sup>1</sup> have shown, however, that the absorptive power of the colloidal clay of different soils may vary widely. Our results show that the content of replaceable bases may bear but little relation to the total clay content, although the replaceable bases reside for the most part in the clay fraction of soils.

During the past ten years K. K. Gedroiz has discussed this subject at considerable length in a series of papers published in the Russian language.\*<sup>11, 12, 13, 14, 15, 16, 17</sup> These papers present by far the most illuminating discussion of the replaceable bases that has come to our attention. We shall make free reference to Dr. Gedroiz' views. In 1922 D. J. Hissink<sup>20</sup> also published an important paper on this subject based on a study of the soils of Holland.†

\* Within the past year English translations of these papers have been secured through the coöperation of Mr. C. S. Scofield of the U. S. Department of Agriculture. The translations were made by Dr. S. A. Waksman of the New Jersey Experiment Station.

† This paper was first published in Dutch in 1920 (Verslag Landbouwk. Onderzoek. Rijkslandbouwproefsta. 24: 144-248). It was republished in German in 1922 in a journal more generally accessible to Americans. An English summary of this paper, prepared by F. H. Smith, was published in 1923,<sup>21</sup> but the serious student will find it helpful to consult the full text. It is noteworthy that Dr. Hissink made no reference to Gedroiz' papers, although practically all the important points which Hissink discussed, with the exception of the electric-double-layer theory, had been considered by Gedroiz previously.

As is well known the amount of the different bases that may be displaced from a soil is related to the concentration of the salt solution used. Gedroiz<sup>12</sup> showed that between certain limits of concentration this is true not only under equilibrium conditions but also when the soluble products of the reaction are continually removed. By repeated extractions with 0.2 N solution of NaCl, KCl or  $\text{NH}_4\text{Cl}$  he found that the replacement was only partial, while normal solutions of any of the common salts will effect complete replacement. However, the rate at which salts of different bases bring about the replacement increases with the atomic weight and valency of the base. Sodium is less energetic in its effect than potassium, while magnesium is more active than potassium but less so than calcium and still less than aluminum and iron. Ammonium and potassium salts are similar in their replacing effect.

When the total content of the several bases present in replaceable form has been substituted by some other base, or when all of the replaceable bases except one have been replaced by a salt of that base, the soil is said to be saturated with respect to that base. Since the replacement reactions are reversible this latter base may in turn be substituted by any other base. These facts are extremely useful in studying this subject. By taking advantage of them it is possible to secure an insight into the significance of the replaceable bases and to determine the total content, inter-relationships and influences of these bases on the various properties of soils.

As will be more fully discussed below it is not entirely certain as yet whether the replaceable bases are chemically combined as true silicates, or whether they are held in some sort of adsorption complex. Nevertheless, it is important to understand that that part of the several bases which is present in replaceable form is quite definite in a given soil and is distinguishable from the remaining portion of the same bases. In fact, soils may contain certain bases in at least four forms. In addition to the replaceable forms, the bases may occur as, (1) water-soluble salts such as chloride, nitrate and sulphate, (2) carbonates and (3) the crystalline silicates and phosphates such as orthoclase, plagioclase, biotite, hornblende, apatite and many other readily distinguishable silicates. The problem dealt with here involves, therefore, the determination of the replaceable bases in the presence of one or more of the other forms.



Our previous investigations have been conducted with special reference to alkali soils. The present paper will be devoted to a consideration of the total content of the several bases present in replaceable form in (1) normal soils of neutral or alkaline reaction, (2) alkali soils, (3) acid soils. A brief discussion of the theoretical aspects of the replacement phenomenon will also be given. In this work we have made free use of the suggestions offered by Gedroiz and Hissink and with the exception of minor points our results have fully confirmed their conclusions.

## I. METHODS

Early in this work it became apparent that  $\text{NH}_4\text{Cl}$  is especially well adapted to the determination of the replaceable bases. The extracts obtained with solutions of this salt may be analyzed with comparatively little difficulty and it has the additional advantage of making possible a simple means of distinguishing between solubility effects and true replacement.

Since the replacement reactions involve an interchange of bases between the soil and the solution, a chemical equivalent of  $\text{NH}_4$  must remain in the soil for every cation brought into solution by replacement. Hence the amount of  $\text{NH}_4$  that is absorbed by the soil is a measure of the total cations replaced. When expressed as chemical equivalents the difference between the absorbed  $\text{NH}_4$  and the total bases in the extract is due for the most part to the solution of soil minerals. With the important exception of soils which contain calcium or magnesium carbonates, we have found no soil in which this difference is of very great magnitude, although solubility effects apparently always affect the results to some extent. The bases originally present in the soil as water-soluble salts, which in alkali soils may be considerable in amounts, must, of course, be subtracted from the amounts found.

That solubility effects as contrasted with replacement have some influence on the results is shown by the fact that the sum of the bases found, expressed as chemical equivalents, usually amounts to a somewhat greater quantity than that represented by the  $\text{NH}_4$  which the soil absorbs. The same conclusion is also indicated by the presence of more or less  $\text{SiO}_2$  in the extract. The extract obtained from every neutral or alkaline soil which we have examined has been found to

contain appreciable amounts of  $\text{SiO}_2$ , amounting in some cases to as much as 0.10 per cent of the soil. This seems to be equally true whether the replacing salt be  $\text{NaCl}$ ,  $\text{KCl}$  or  $\text{NH}_4\text{Cl}$ . With soils of coarse type such as sandy soils the amount of bases that is merely dissolved rather than replaced, although small in absolute quantity, may nevertheless constitute a considerable percentage of the total bases found. This seems to be especially true of coarse soils of comparatively recent geological origin. For this reason heavy types of soil are best suited to a study of the replaceable bases. However, we have made the determination successfully with several sandy loam soils.

The method we have used is with minor modifications a combination of that recommended by Hissink<sup>20</sup> and one of those proposed by Gedroiz.<sup>15</sup> The procedure was as follows: Twenty-five gm. of air dried soil and 250 cc.  $\text{NH}_4\text{Cl}$  solution were placed in a flask, shaken by hand and then held over night in an oven kept at  $70^\circ \text{C}$ . The following morning the contents of the flask were thrown on a folded filter. After the solution had drained through the filter the soil remaining in the flask was transferred to the filter and the residue was leached with successive portions of  $\text{NH}_4\text{Cl}$  solutions until 1000 cc. of filtrate was obtained.

Eight hundred cc. of the filtrate was transferred to a large porcelain dish and concentrated to a small volume on a water bath. Fifty to 75 cc. strong  $\text{HNO}_3$  was then added and the evaporation continued to dryness. By this means the  $\text{NH}_4\text{Cl}$  was decomposed and a residue obtained consisting of nitrates of the bases extracted from the soil together with small amounts of  $\text{SiO}_2$ . The residue was brought to dryness two or three times after adding strong  $\text{HCl}$  in order to convert the nitrates into chlorides and dehydrate the silica. After removing the  $\text{SiO}_2$  by filtration, the bases were determined by the use of standard methods of chemical analysis.

The soil residue remaining after the extraction with  $\text{NH}_4\text{Cl}$  was leached with distilled water until free from  $\text{Cl}$ . It was then transferred to a flask, 400 cc.  $\text{H}_2\text{O}$  and 50 cc. strong  $\text{NaOH}$  solution were added and the  $\text{NH}_4$  determined by distillation.

All determinations were made in duplicate and certain of them were repeated several times. Closely concordant results were almost always obtained.



In studies on methods we used a clay loam soil of the Ramona series (431), on which considerable experimental data have already been published from this laboratory.<sup>7, 22</sup> The soil is almost exactly neutral, contains no carbonate and is practically free from water-soluble salts.

#### EFFECT OF CONCENTRATION OF $\text{NH}_4\text{Cl}$

The results reported in table 1 show that both the total amount of bases extracted and the  $\text{NH}_4$  absorbed increased with increasing concentrations of  $\text{NH}_4\text{Cl}$  up to a certain concentration above which the results were fairly constant. These data indicate that the soil contains fairly definite amounts of replaceable bases and that complete replacement requires the use of a concentration of  $\text{NH}_4\text{Cl}$  slightly more than one-half normal. With concentrations greater than normal the increased amount of the bases found, which was mainly calcium, seems to be due entirely to solubility effects. It is noteworthy that K and Na may be more readily replaced than the divalent bases and Mg somewhat more so than Ca. These results agree with those of Gedroiz and Hissink.

TABLE 1

EFFECT OF CONCENTRATION OF  $\text{NH}_4\text{Cl}$  ON THE DETERMINATION OF REPLACEABLE BASES

Concentration of solution	Per cent of soil				Milligram equivalents	
	Ca	Mg	K	Na	Total bases	$\text{NH}_4$ absorbed
N/32.....	0.240	0.055	0.048	0.013	18.4	17.0
N/16.....	0.315	0.061	0.050	0.017	22.8	21.3
N/8.....	0.375	0.069	0.039	0.018	26.2	24.2
N/4.....	0.408	0.072	0.039	0.019	28.2	26.2
N/2.....	0.423	0.073	0.041	0.021	29.1	27.1
N/1.....	0.436	0.073	0.053	0.028	30.4	27.3
2N.....	0.453	0.078	0.051	0.028	31.6	27.5
3N.....	0.453	0.076	0.051	0.027	31.4	27.4

As a check on the method for the determination of absorbed  $\text{NH}_4$  the soil residue was freed from  $\text{NH}_4\text{Cl}$  by leaching with 80 per cent alcohol. In other cases the  $\text{NH}_3$  was driven out of the soil by the use of an alkaline solution of normal  $\text{CaCl}_2$ . The results were quite similar

to those reported above. As further evidence on the accuracy of the method other portions of the soil were completely saturated with K and Ca by leaching with solutions of KCl or  $\text{CaCl}_2$ . The soil residue was then leached free from Cl and the replaceable K and Ca were determined by treatment with a normal solution of  $\text{NH}_4\text{Cl}$  as described above. The results expressed as milligram equivalents per 100 gm. soil were 27.1 for K and 27.2 for Ca, while the  $\text{NH}_4$  absorbed as determined by distillation was 27.3. Thus it is shown that the  $\text{NH}_3$  obtained by distillation gives an accurate measure of the total bases replaced. These data further show that the replacement takes place by chemical equivalents, whether K, Ca or  $\text{NH}_4$  salts are used.\*

### RATIO OF SOIL TO SOLUTION

The volume of solution with which it is necessary to leach a given quantity of soil in order to effect complete replacement was also studied. In each case 25 gm. of soil and 250 cc. normal  $\text{NH}_4\text{Cl}$  were held over night at  $70^\circ \text{C}$ . Duplicate portions were then leached with  $\text{NH}_4\text{Cl}$  solution until 500 cc., 1000 cc. and 2000 cc. of leachate were collected. The results (table 2) show that substantially the same amount of replacement took place in each case. As might be expected, however, solubility effects became more pronounced with the more prolonged leaching (compare the total base found with the  $\text{NH}_4$  absorbed).

TABLE 2  
RESULTS OBTAINED BY LEACHING WITH DIFFERENT VOLUMES OF NORMAL  
 $\text{NH}_4\text{Cl}$  SOLUTION

Volume of solution	Per cent of soil				Milligram equivalents	
	Ca	Mg	K	Na	Total bases	$\text{NH}_4$ absorbed
500 cc.....	0.415	0.070	0.043	0.026	28.7	27.5
1000 cc.....	0.436	0.073	0.053	0.028	30.4	27.3
2000 cc.....	0.456	0.075	0.053	0.032	31.8	27.4

\* Milligram equivalent signifies chemical equivalents expressed in milligrams and hence its determination involves a consideration of the atomic weight and valency. The data were obtained by dividing the milligrams of a given base found per 100 gm. of soil by its atomic weight and multiplying the quotient by the valency of the base. For example, 100 gm. of this soil when saturated with Ca contains 544 milligrams of replaceable Ca.  $\frac{544}{40} \times 2 = 27.2 \text{ M.E.}$

## RATE OF REPLACEMENT

Gedroiz and Hissink have called attention to the remarkable speed of the replacement reactions. In their work on this point they employed equilibrium conditions, under which complete replacement does not usually take place. It seemed desirable to determine the effect of varying the time of digestion with  $\text{NH}_4\text{Cl}$  solution before filtering and leaching the residue. The experiments were made with normal  $\text{NH}_4\text{Cl}$  at room temperature instead of at  $70^\circ \text{C}$ . as before.

It will be observed (table 3) that contact between the soil and the  $\text{NH}_4\text{Cl}$  solution for a period of ten minutes, followed by the time necessary to secure 1000 cc. of filtrate, effected almost as great replacement as contact for 16 hours. It is probable that merely leaching this soil with no preliminary shaking will give approximately complete replacement. By referring to the preceding experiments it will be noted, however, that lower results were obtained at room temperature than at  $70^\circ \text{C}$ .

TABLE 3  
EFFECT OF TIME ON THE REPLACEMENT OF BASES

Time of contact	Per cent of soil				Milligram equivalents	
	Ca	Mg	K	Na	Total bases	$\text{NH}_4$ absorbed
10 minutes.....	0.372	0.060	0.030	0.013	24.9	23.4
30 " .....	0.384	0.059	0.034	0.014	25.6	23.6
60 " .....	0.386	0.062	0.032	0.016	25.9	23.1
3 hours.....	0.403	0.072	0.041	0.025	28.2	24.4
6 " .....	0.397	0.064	0.050	0.019	27.2	24.2
16 " .....	0.400	0.073	0.047	0.020	28.1	24.1

Since an important object of this work was to determine the total content of the replaceable bases, all the remaining data were obtained by first digesting 25 gm. of the soil with 250 cc. normal  $\text{NH}_4\text{Cl}$  solution for several hours at  $70^\circ \text{C}$ ., and then leaching to one liter. It is probable, however, that a more rapid method and one requiring less extravagant use of  $\text{NH}_4\text{Cl}$  will give accurate results.

EFFECT OF  $\text{CaCO}_3$ 

When calcium carbonate is present difficulties arise in the determination of the replaceable Ca for the reason that the carbonate is soluble to some extent in solutions of all the common salts and markedly soluble in  $\text{NH}_4\text{Cl}$  solutions. Hissink attempted to avoid this difficulty by extracting with two successive liters of normal NaCl solution. He considers the Ca found in the first liter minus that in the second to be a measure of the total replaceable Ca. The theoretical basis for this method rests on the assumption that as much  $\text{CaCO}_3$  will be dissolved by the first liter as by the second, but since replacement takes place extremely rapidly and since the solubility of  $\text{CaCO}_3$  is repressed by the presence of Ca ions in the solution the results obtained by Hissink's method may be a little low.

Gedroiz<sup>15</sup> recommended the determination of the total  $\text{CO}_2$  in the soil before and after extracting with  $\text{NH}_4\text{Cl}$  solution. From the data thus obtained he introduced a correction on the assumption that for each mol of  $\text{CO}_2$  removed from the soil one mol of  $\text{CaCO}_3$  was dissolved.

TABLE 4  
EFFECT OF  $\text{CaCO}_3$  ON THE DETERMINATION OF REPLACEABLE BASES

CaCO <sub>3</sub> added	Per cent of soil				Milligram equivalents	
	Ca*	Mg	K	Na	Total bases	NH <sub>4</sub> absorbed
None.....	0.436	0.073	0.053	0.028	30.4	27.3
0.5 per cent.....	0.436	0.073	0.053	0.028	30.4	28.0
1.0 " ".....	0.434	0.072	0.052	0.027	30.2	28.0
2.0 " ".....	0.392	0.071	0.053	0.028	28.1	28.0
4.0 " ".....	0.315	0.072	0.053	0.028	24.3	23.2

\* The Ca data were corrected for the  $\text{CaCO}_3$  dissolved by the  $\text{NH}_4\text{Cl}$  solution.

The data in table 4 show the results obtained where varying amounts of  $\text{CaCO}_3$  were added. The determinations were made in the manner already described, with the additional determination of  $\text{CO}_2$  in the soil after extracting with  $\text{NH}_4\text{Cl}$  solution. Corrections were made in the Ca determinations for the  $\text{CaCO}_3$  dissolved. Where the amount of  $\text{CaCO}_3$  present was not more than 1.0 per cent of the

soil the results were quite accurate, but with higher percentages not all of the replaceable Ca was obtained. In still other experiments we have found, as pointed out by Gedroiz, that it is necessary to continue the extraction until practically all of the  $\text{CaCO}_3$  was dissolved. Then by making a correction for  $\text{CaCO}_3$  the content of replaceable Ca can be calculated. It is true, however, that prolonged extraction with  $\text{NH}_4\text{Cl}$  tends to dissolve increased amounts of bases combined in non-replaceable forms. As yet we have not found an entirely satisfactory method for the determination of replaceable Ca in the presence of high percentages of  $\text{CaCO}_3$ .

Where  $\text{CaCO}_3$  and  $\text{MgCO}_3$  occur the difficulties are still greater. Fortunately soils which contain both of these carbonates are not common, although we have found one such case. The presence of insoluble carbonates does not interfere with the determination of replaceable K and Na.

## II. NORMAL SOILS OF NEUTRAL OR ALKALINE REACTION

The following soils were studied: sample 302, Yolo loam which contains 0.102 per cent  $\text{CaCO}_3$  taken from Santa Paula, California; 430, Placentia sandy loam free from carbonate, from the Citrus Experiment Station Farm, Riverside, California; 431, Ramona clay loam free from carbonate taken from La Habra, California; 529, Porterville clay loam which contains 1.395 per cent  $\text{CaCO}_3$  taken near Lindsay, California; 539, Chino clay loam with 0.904 per cent  $\text{CaCO}_3$  from Spadra, California; 2767, Yolo loam containing 0.250 per cent  $\text{CaCO}_3$  from Tustin, California; 6274, Olympic clay loam free from carbonate taken near Lemon Cove, California.

The data shown in table 5 have been corrected for water-soluble salts and  $\text{CaCO}_3$ . It will be seen that soil 430, the lightest type studied, contained by far the least amount of replaceable bases, and soil 302, the next lightest type, contained considerably less than the other samples. None of them contained more than traces of replaceable Al, Fe or Mn.



TABLE 5  
REPLACEABLE BASES IN NEUTRAL OR SLIGHTLY ALKALINE SOILS

Soil	Per cent of soil				Milligram equivalents		Relative proportion of bases			
	Ca	Mg	K	Na	Total bases	NH <sub>4</sub> absorbed	Ca	Mg	K	Na
430.....	0.083	0.016	0.017	0.021	6.7	4.6	61.2	19.4	6.0	13.4
431.....	0.436	0.073	0.053	0.028	30.4	27.3	71.7	20.1	4.3	3.9
302.....	0.250	0.051	0.046	0.045	19.8	14.4	63.1	21.2	6.1	9.6
520.....	0.655	0.136	0.034	0.049	47.0	*	69.6	24.0	1.9	4.5
539.....	0.635	0.176	0.037	0.062	49.9	44.1	63.5	29.3	1.8	5.4
2767.....	0.269	0.097	0.021	0.073	25.1	24.1	53.4	32.3	2.0	12.3
6274.....	0.401	0.127	0.039	0.043	33.5	28.5	59.7	31.6	3.0	5.7

\* Not determined.

When the results are expressed not as percentages of the soil but as percentages of the total replaceable bases, calculated as chemical equivalents, a different picture is presented. In every soil Ca comprised more than 50 per cent of the total bases found and Mg 20 or more per cent, whereas the replaceable K and Na were low, both absolutely and relatively. In each soil the sum of the divalent bases equalled 80 or more per cent of the total. These results are of special interest in connection with the alkali soils discussed below.

It is of interest to compare these California soils with the European soils reported by Gedroiz and Hissink.

	Relative percentage of replaceable bases			
	Ca	Mg	K	Na
California soils (av. 7 samples).....	63	25	4	8
Russian soils (Gedroiz, av. 2 samples).....	82	11	7	0
Holland soils (Hissink, av. 26 samples).....	79	13	2	6

It will be noted that the California soils contain relatively less replaceable Ca and greater amounts of replaceable Mg than the soils reported by Gedroiz and Hissink, but when the magnitude of the analytical error and the solubility effects are taken into consideration the relative proportions of K and Na are not greatly different. The total quantity of replaceable bases found in the several European soils differed quite widely. This was especially true with the two Russian



soils, one of which was a podsol and the other a tshernoziem, but they appear to be remarkably similar qualitatively.

The origin of the California soils is known fairly definitely, especially so in the case of soils 430, 431, 520, 2767 and 6274. Certain of them have been derived from granite, others from shales and sandstones which themselves were derived from granite and still others from hornblende gabbro. The origin of the European soils on the other hand is unknown to us; they probably came from mixed sources.

On the basis of these data there does not appear to be any fundamental difference in the California soils which can be traced to the minerals from which they were derived. These soils are probably much more recent geologically than those reported by Gedroiz and Hissink, a fact which may have some bearing on the variations noted in the ratio of replaceable Ca to Mg. Further reference will be made to this point later.

The importance of the fact that Ca and Mg comprise a very large percentage of the total replaceable bases in normal soils will become more evident when we consider alkali and acid soils.

### III. ALKALI SOILS

The following alkali soils have been investigated: sample 1869, Fresno fine sandy loam from the Kearney Vineyard, Fresno, California; 5190, Jordan fine sandy loam drawn near Salt Lake, Utah; 5696, Lahontan clay from Fallon, Nevada; 6145, silty loam from the University Farm, Tucson, Arizona; 6155, Hanford silty loam from Arlington, California. These soils contained relatively high concentrations of soluble salts either when sampled or within a comparatively recent period. The soluble materials consisted mainly of Na salts. In addition to NaCl and  $\text{Na}_2\text{SO}_4$  all of these samples except No. 6155 contained considerable  $\text{Na}_2\text{CO}_3$ . With the exception of soil 1869 substantial amounts of  $\text{CaCO}_3$  were present, and soil 5190 also contained  $\text{MgCO}_3$  or  $\text{CaMg}(\text{CO}_3)_2$ .

It will be observed (table 6) that the replaceable bases of these soils bear an altogether different relationship from that found in normal soils. Instead of Ca and Mg comprising a high percentage of the total, as in normal soils, all of these samples contained no replaceable Ca and with the exception of 6155 none of them contained replaceable Mg.

TABLE 6  
REPLACEABLE BASES IN ALKALI SOILS

Soil	Per cent of soil				Milligram equivalents		Relative proportion of bases			
	Ca	Mg	K	Na	Total bases	NH <sub>4</sub> absorbed	Ca	Mg	K	Na
1869.....	0	0	0.084	0.091	6.0	3.0	0	0	35.0	65.0
5190.....	0	0	0.143	0.137	9.5	7.0	0	0	39.6	60.4
5696.....	0	0	0.031	0.570	25.6	24.0	0	0	3.1	96.9
6145.....	0	0	0.072	0.174	9.4	5.2	0	0	19.1	80.9
6155.....	0	0.080	0.057	0.094	12.2	12.3	0	54.1	12.3	33.6

As stated above considerable  $\text{Na}_2\text{CO}_3$  was present in every sample except 6155, and the amount was sufficient to maintain a highly alkaline soil solution and thus precipitate as carbonates any Ca or Mg that may have been brought into solution by the replacing action of Na or otherwise. The soluble products of the reaction being thus removed from solution, the replacement of the divalent bases must have already proceeded to completion or approximately so. The presence of more or less  $\text{CaCO}_3$  in alkali soils is probably due in part at least to its formation through these reactions.

It should not be inferred from these data that all alkali soils are free from replaceable Ca. This will depend on the concentration of the soluble salts together with the presence or absence of alkali carbonates. If the concentration be comparatively low only a part of the replaceable Ca will be substituted by other bases. Undoubtedly alkali soils frequently occur which contain more or less replaceable Ca, but with the important exception of those cases where a considerable amount of soluble Ca salts occur, at least a part of the replaceable Ca must be substituted by other bases. The results are in harmony with Gedroiz' data on the saline soils of Russia and with those of Hissink on the polder soils of Holland.

In considering alkali soils it is important to bear in mind that the relative proportions of the different bases present in replaceable form at a given time may reflect, in some measure at least, the effects of soluble salts which were in contact with the soil at some period previous to the time of sampling as well as of those present when the sample is drawn. It is probable that the kind and amounts of soluble salts in a

given place vary from time to time owing to the vicissitudes of climate, the methods used in handling the soil, etc. At one period a high concentration may prevail and later this may be greatly reduced by the leaching action of rains and still later the composition of the salts may be changed as a result of the deposition of soluble salts from other places. The result will be that the relative proportions of the replaceable bases may fluctuate.

Soils 5190 and 5696 represent for the most part lacustrine materials which were deposited in saline water. The water finally drained away or evaporated and rains have leached out most of the salts, particularly in soil 5696. The result is that these soils now contain no replaceable Ca or Mg. The relatively high content of replaceable Mg and K in certain of the samples was probably brought about by the action of soluble K and Mg salts. High concentrations of salts of these bases occur at the present time in various places in western America.

As has been pointed out by several investigators the deflocculated condition which develops in many alkali soils upon leaching out the excess of soluble salts is one of their most pronounced characteristics. This condition frequently develops where little if any  $\text{Na}_2\text{CO}_3$  is present but is especially evident in black-alkali soils. A determination of the replaceable bases in samples drawn from certain so-called "slick spots" which are characterized by pronounced impermeability, has shown a high content of replaceable Na. Soil 5696 is an extreme example of this condition. This sample typifies a comparatively large area whose soluble-salt content is not exceptionally high at present, but upon which scarcely a vestige of plant life can be found. Fundamentally the toxicity of this soil is due to its high content of replaceable Na rather than to soluble salts as such. As will be shown in a later paper soils whose replaceable base consists mainly of Na may be extremely toxic.

The studies that have been made on this subject show quite clearly that the fundamental cause of the deflocculated condition in these and other alkali soils lies in the nature of the replaceable bases. Whenever Na constitutes any considerable percentage of the total replaceable bases the soil upon leaching out the excess of soluble salts is almost certain to manifest colloidal properties in high degree. It is important to understand, however, that is not the absolute amount of replaceable Na but rather its relation to the replaceable divalent bases, Ca in

particular, that determines whether the soil will be excessively colloidal. We expect to develop this subject more fully in a separate paper.

In the practical treatment of alkali soils the soluble salts must, of course, be dealt with, but this is by no means the only consideration. Certain types of alkali soils will certainly not become normal or productive merely through removal of the excess of soluble salts as has so often been assumed in the literature of alkali soils and in reclamation practice. The replaceable Na must also be displaced before the soil can be said to be fully reclaimed. Unless this be done the efforts and expense devoted to drainage and leaching will, in some cases at least, avail little or nothing.\* As shown above the content of replaceable Na both absolute and relative varies widely in different soils. Other things being equal it seems safe to assert that the lower the content of replaceable Na the more readily may an alkali soil be reclaimed. In the present state of knowledge it seems doubtful whether a soil whose total content of replaceable Na is as high as that of soil 5696 can be reclaimed economically. The difficulty becomes still greater where both the soil and the subsoil contain a high content of replaceable Na.

Three of the above samples, Nos. 1869, 5190 and 6145, were taken from areas on which reclamation experiments are in progress. The results thus far obtained are by far the most successful in the case of soil 5190. Certain treatments applied to soil 6145 have also yielded encouraging results, while with soil 1869 almost identically the same kinds of treatments that have been used on 5190 and 6145 have yielded mediocre results. It will be noted that the replaceable bases are not greatly different in these soils, either quantitatively or qualitatively. These soils differ very greatly, however, in their content of  $\text{CaCO}_3$ . Soil 1869 contains a very low amount; soil 6145 a considerable amount and 5190 a very high amount. Our studies show that a given soil when saturated with Na becomes more permeable upon adding  $\text{CaCO}_3$  in excess. Calcium carbonate tends to be dissolved by the soil moisture, especially in the presence of decaying organic matter, and in consequence it promotes a gradual reversal of the replacement process, thus building up a system in which Ca has displaced the Na. If leaching conditions be maintained as has been the case with the practical experi-

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\* Scofield has recently published a method by which the combined water-soluble and replaceable Na may be determined.<sup>32</sup>



ments on soil 5190 the replaceable Na will give way to Ca, the former being leached out as  $\text{NaHCO}_3$  and in the course of time a soil system will result in which the replaceable bases will become normal. This view is also in harmony with the opinion of Gedroiz and Hissink.

Another important practical consideration is found in the composition of the irrigation water. That which has been used in the field experiments on soil 1869 contains about 50 parts per million total salts which are composed mainly of sodium compounds. On the other hand the water used to irrigate soil 5190 contains approximately 1000 p. p. m. in which the ratio of the monovalent to divalent bases is somewhat less than 2. The water applied to soil 6154 is intermediate between the other two both in concentration and composition.

Thus it appears that a high content of  $\text{CaCO}_3$  and an irrigation water relatively rich in Ca and Mg salts are favorable to the reclamation of alkali soils. In such cases a Na saturated soil may be comparatively easily converted into a soil with normal properties, whereas in the absence of one or both of these conditions the reclamation may be difficult.

#### ALKALI SOILS WHICH CONTAIN SOLUBLE Ca SALTS

Many alkali soils contain considerable amounts of soluble Ca salts in addition to Na salts. We have determined the replaceable bases in two such soils (table 7). Although these soils contained large amounts of soluble salts, a considerable part of which was Na compounds, the relative amounts of the replaceable bases were found to be similar to those of normal soils. The reason for this must be apparent from the preceding discussion. The soluble Ca salts were sufficiently high to prevent the replacing action of Na. Where the amount of soluble Ca in proportion to Na is sufficiently great Na is not able to replace other bases. With low total concentrations small amounts of Ca may be replaced when the ratio of Ca to Na is as 1:2; whereas with high concentrations the ratio of divalent to monovalent bases may be as 1:5 or more and still no replacement will take place.

TABLE 7  
REPLACEABLE BASES IN SOILS WHICH CONTAIN SOLUBLE SODIUM AND  
CALCIUM SALTS

Soil	Per cent of soil				Milligram equivalents		Relative proportion of bases			
	Ca	Mg	K	Na	Total bases	NH <sub>4</sub> absorbed	Ca	Mg	K	Na
6157.....	0.380	0.162	0.040	0.047	35.5	31.2	53.6	38.0	2.8	5.6
6158.....	0.333	0.074	0.014	0.019	23.8	22.5	69.7	25.7	1.3	3.3

Upon leaching out the excess of soluble salts these two soils became pulverent and manifested properties quite similar to those of normal soils. The extreme deflocculation referred to above was not manifest. Such soils are readily amenable to reclamation by simple drainage and flooding. A considerable portion of the alkali areas of California is probably of this nature.

#### IV. ACID SOILS

The following acid soils were studied: 3232, Clermont silt loam from North Vernon, Indiana; 6251, a clay loam of an unclassified series from Mendocino County, California; 6275, Rhonerville clay loam from Rhonerville, California; 6276, Hagerstown clay loam from the Pennsylvania Experiment Station; 6277, Melbourn clay loam from western Oregon; 6278, Greenville clay loam from South Carolina. The results are recorded in table 8.

The content of replaceable Ca, Mg, K and Na was extremely low in these soils as compared with that of the neutral or alkaline soils of similar texture. The average total amount of these bases in the acid soils was 4.1 milligram equivalents and in the non-acid soils 29.6 milligram equivalents. Each of the acid soils contained more or less replaceable Al and Mn and two of them replaceable Fe. When expressed on the basis of chemical equivalents the trivalent bases were found to comprise a considerable percentage of the total. In fact the sum of the trivalent bases was in excess of the combined monovalent and divalent bases in soil 6276 and was almost as great in soils 6275 and 6277. The NH<sub>4</sub> absorbed was approximately equal to the sum of all the bases found.





On the basis of these data there appears to be a fundamental difference both qualitative and quantitative between the replaceable bases of acid and those of non-acid soils. It should not be inferred, however, that acid soils necessarily contain a low content of replaceable bases or replaceable Al, Fe or Mn.\* As will be shown in the following section of this paper, a neutral soil may become acid upon treatment with carbonated water. In this case only a small part of the bases need be substituted by hydrogen in order to make the soil distinctly acid.

It has long been known that an acid solution is obtained by shaking certain soils with a neutral solution of K or Na salts. The Hopkins method for the determination of soil acidity is based on this fact. Veitch<sup>39</sup> and others have explained this fact on the basis of replaceable trivalent bases. There is considerable difference of opinion, however, as to whether the acidity is due to the presence of hydrolyzable salts of the trivalent bases, or whether the trivalent bases are actually replaced by the salt. Comber has proposed a qualitative test for acid soils based on the amount of Fe brought into solution by KCNS<sup>5</sup> or potassium salicylate.<sup>6</sup> He holds that Fe is replaced by K of these salts. It does not follow, however, that the acidity of soils in general is due entirely to salts of Fe or to replaceable Fe. Our data show that the content of replaceable Fe is not always proportional to the total content of replaceable trivalent bases and that a soil may be decidedly acid without containing more than traces of replaceable Fe.

It may be pointed out that the benefits to be derived from the application of lime appear to be determined not solely by the pH value of the soil, but also by the composition, concentration and the buffer properties of the soil solution. These latter are determined by the solubility of constituents not involved in replacement as well as by the readiness with which the replaceable bases are brought into solution. It is probable, however, that the crop-producing power of any soil whose content of replaceable Ca is low and which does not contain  $\text{CaCO}_3$  will be increased by the application of lime. This is

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\* Since this manuscript was written we have examined four other acid soils sent to us from widely separated localities east of the Mississippi River. Each of these samples contains substantial amounts of replaceable Al, Fe and Mn. Their total content of replaceable bases is also relatively low. Two of them are quite similar to the acid soils reported herein both qualitatively and quantitatively. The others contain somewhat greater amounts of replaceable bases, but neither is strongly acidic.

especially true when legumes or other crops which have a high Ca requirement are grown. The constituents concerned in basic exchange are the most labile and reactive components of soils. They are probably important sources from which the soil solution derives its Ca content.

Gedroiz and Hissink have pointed out that favorable physical properties are promoted by a high content of replaceable Ca in proportion to the other replaceable cations. As will be shown later the physiological properties as determined by culture experiments may be profoundly modified by alterations in the normal ratio of these bases. When  $\text{CaCO}_3$  is absent a considerable amount of replaceable Ca seems to be one of the essentials for the sustained production of high yields of crops. The content of replaceable Ca, therefore, constitutes an important characteristic of soils.

Hissink showed that a part of the replaceable Ca can be easily removed from soils by extraction with carbonated water. He holds that the soil when so treated becomes unsaturated with bases, that is, hydrogen ions of the carbonated water displace the bases from the soil. He believes that soils tend towards this condition in humid climates. Many Holland soils are only partially saturated with bases. Hissink found that soils which contain similar amounts of clay may differ materially in their total content of replaceable bases. This fact is attributed to unsaturation in the sense just mentioned. He proposed a scheme for expressing the degree of saturation by designating the sum of the replaceable bases as (S) and the total capacity for bases as (T). The difference between (T) and (S) expressed as chemical equivalents represents the quantity of base required to bring the soil to complete saturation. Gedroiz believes that the processes involved in the formation of podsol soils likewise involve the substitution of hydrogen, derived from meteoric waters and decaying organic matter, in the place of more or less of the replaceable bases. He further holds that the substituted hydrogen can itself be replaced by bases, but only, however, with much greater difficulty than can the replaceable bases.

Bradfield<sup>2</sup> has shown that colloidal clays separated from different soils have the power to neutralize different amounts of dilute alkaline solutions. In other words they possess different degrees of acidity. He also presented strong evidence that  $\text{Al}_2\text{O}_3$  and  $\text{SiO}_2$  present in the colloidal material of an acid soil occur in chemical combination as

alumino silicate.<sup>3, 4</sup> It is possible that the colloidal alumino silicates of different soils may contain variable amounts of bases, and if so the acidity may be due to substituted hydrogen or unsaturation in the sense of Gedroiz and Hissink. Gile and his associates<sup>19</sup> have found that the colloidal clay materials of different soils have the power of absorbing  $\text{H}_2\text{O}$ ,  $\text{NH}_3$  and malachite green in quite variable amounts. In the case of the dye the differences found range from 0.0584 gm. to 0.4128 gm. per gram of the colloid.

#### ABSORPTION OF $\text{Ca}(\text{OH})_2$

Samples of the acid soils reported in table 8 were digested for several hours at  $70^\circ \text{C}$ . with 0.04N  $\text{Ca}(\text{OH})_2$  and then thoroughly leached with  $\text{Ca}(\text{OH})_2$  solution. They were then washed with distilled water in order to remove the excess of  $\text{Ca}(\text{OH})_2$ . The wash water at first became strongly alkaline showing that an excess of  $\text{Ca}(\text{OH})_2$  had been used, and in most cases it continued to be alkaline after prolonged leaching. After the alkalinity had been reduced to a low level and practically all of the water-soluble Ca had been removed, the soil was treated with normal  $\text{NH}_4\text{Cl}$  exactly as was done originally in the determination of replaceable bases.

TABLE 9

BASES EXTRACTED BY  $\text{NH}_4\text{Cl}$  AFTER FIRST TREATING THE SOIL WITH  $\text{Ca}(\text{OH})_2$

Soil	Per cent of soil						Milligram equivalents	
	Ca	Mg	K	Na	Al	Fe	Total bases	$\text{NH}_4$ absorbed
431.....	.958	.043	.044	.039			54.2	27.4
3232.....	.378	.012	.012	.029	*	*	21.4	6.9
6251.....	.199	.017	.011	.019	*	*	12.4	3.2
6275.....	.766	.011	.011	.016			40.2	16.7
6276.....	.564	.006	.004	.009	.009	.013	30.9	10.4
6277.....	.892	.007	.003	.015	.003	.004	47.1	20.7
6278.....	.437	.008	.002	.013	.003		23.4	3.5

\* Not determined.

By comparing the data shown in tables 8 and 9 it will be observed that the treatment with  $\text{Ca}(\text{OH})_2$  greatly increased the amount of Ca that was capable of being brought into solution with  $\text{NH}_4\text{Cl}$ . The Ca

thus absorbed is soluble in  $\text{NH}_4\text{Cl}$  solution, but not necessarily as a result of replacement of bases as indicated by the  $\text{NH}_4$  data. Similar results were obtained when the treated soil was extracted with  $\text{NaCl}$  instead of  $\text{NH}_4\text{Cl}$ .

The replaceable base content of soils 6251 and 6278 both of which are heavy clays was still extremely low after treatment with  $\text{Ca}(\text{OH})_2$ . As yet we have found no evidence that their content of replaceable base can be increased by any simple treatment. After treatment with  $\text{Ca}(\text{OH})_2$  certain of these soils still contained Al and Fe soluble in  $\text{NH}_4\text{Cl}$ . It is of special interest that the total amount of replaceable bases, as measured by the amount of  $\text{NH}_4$  absorbed, was not increased in certain of the acid soils, while in others it was markedly increased. Soils 6275, 6276 and 6277 apparently contain replaceable hydrogen, whereas the other acid soils do not. With the former a part of the absorbed Ca apparently entered into replaceable form analogous to that normally present. In this case the Ca probably replaced hydrogen, but the capacity of soils to absorb  $\text{Ca}(\text{OH})_2$  is not limited by their content of replaceable hydrogen or other cations. For example, the neutral soil 431 discussed above has the power to absorb greater amounts of  $\text{Ca}(\text{OH})_2$  than any acid soil that we have studied. Other neutral soils are also able to absorb notable amounts of  $\text{Ca}(\text{OH})_2$ , but the absorbed Ca does not enter into replaceable form.

## V. SPECIAL EXPERIMENTS AND DISCUSSION

Gedroiz<sup>16, 17</sup> holds that the replaceable bases occur either as humates or in zeolitic forms, the latter predominating in mineral soils. He uses the term "zeolite" not in the strict sense of the mineralogist, but because of the similarity between the properties of mineral soils and true zeolites. He suggests three possible modes of formation and origin of the so-called zeolitic constituents: (1) comminution by mechanical agencies of the minerals from which the soil has been derived, (2) formation of secondary silicates as a result of the weathering of the primary minerals, (3) colloidal precipitation of  $\text{SiO}_2$  and  $\text{Al}_2\text{O}_3$  occasioned by the opposite charge which these colloids bear. The colloidal complex thus formed absorbs the bases which take part in replacement. While Gedroiz states that all three of these processes may be involved to some extent, he considers the last named to be the



most important. His idea of the nature of the union between the bases and the colloidal complex is not entirely clear, however, nor why it is that such a colloidal complex is better able to absorb bases than colloidal silica or other electro-negative colloids.

CLAY AND THE COARSE FRACTION OF SOILS

By the use of the method outlined by Anderson, *et. al.*,<sup>1</sup> we have separated a considerable quantity of the so-called ultra-clay from three normal soils of neutral or only slightly alkaline reaction. The replaceable bases were then determined in this material. The soils from which the clay was separated contained widely different amounts of clay material. As shown in table 10 the clays also differed materially in their content of replaceable bases, although the clay from each soil contained a much higher percentage than the soil from which it was separated (compare tables 5 and 10).

TABLE 10  
REPLACEABLE BASES IN THE CLAY AND COARSE MATERIALS OF SOILS

Materials	Per cent				Relative proportion of bases			
	Ca	Mg	K	Na	Ca	Mg	K	Na
430 - Clay.....	0.502	0.100	0.065	0.062	66.6	22.0	4.2	7.2
431 - Clay.....	.978	.209	.135	.033	66.4	26.3	5.1	2.1
520 - Clay.....	1.104	.217	.069	.085	70.0	23.0	2.3	4.7
430 > 100 mesh.....	.024	.026	.032	.032	21.4	39.3	14.3	25.0
431 > 100 mesh.....	.070	.039	.049	.062	33.0	30.2	11.3	25.5
430 > 100 mesh ground < 200 mesh.....	.030	.026	.041	.045	22.4	32.8	14.9	29.9
431 > 100 mesh ground < 200 mesh.....	.089	.040	.053	.069	36.7	27.5	10.8	25.0

The part of two of these soils which would not pass a 100-mesh sieve was also studied both with and without grinding to pass a 200-mesh sieve. The grinding undoubtedly reduced a part of the coarse material to colloidal dimensions. It will be noted that the content of replaceable bases was quite low in both cases and that the grinding produced very little effect on replacement. A considerable part of the bases found was probably brought into solution as a result of solubility



effects rather than by replacement. It will be noted also that the relative proportions of the several bases extracted from the coarse fractions were quite different from those found in the clay materials. The coarse materials were composed very largely of feldspars, hornblende, mica, etc., the minerals from which these soils have been derived.

Other experiments were made in which the whole soil was ground fine enough to pass a 200-mesh sieve, but the grinding produced no appreciable effect on the replaceable bases.

These experiments show that the replaceable bases reside mainly in the finer fractions of these soils, probably in the clay, as has long been held to be the case. They indicate further that the bases have not become replaceable as a result of the mere comminution of the primary minerals.

TABLE 11  
REPLACEABLE BASES IN MATERIALS FROM WHICH SOILS HAVE BEEN DERIVED

	Per cent				Relative proportion of bases			
	Ca	Mg	K	Na	Ca	Mg	K	Na
Hornblende gabbro.....	0.095	0.018	0.009	0.004	71.2	22.7	3.0	3.0
Shale.....	.491	.090	.087	.058	66.8	20.4	6.0	6.8

In contrast to the preceding results we have found that the replaceable bases of a clay soil of the Olympic series occur in relative proportions quite similar to those of the finely ground hornblende gabbro (table 11) from which this soil has been derived. However, the total content of replaceable bases in the former was very much greater than in the latter. A sample of argillaceous shale free from  $\text{CaCO}_3$  and ground to pass a 200-mesh sieve was also studied. Soil 539, reported in table 5, has been derived in part from the formation from which the sample of shale was obtained. The shale was relatively rich in replaceable bases and the bases were present in relative proportions quite similar to those of the soil nearby.

It was also found that heating a soil to 110° C. for a period of 30 hours produced no effect on the replaceable bases. These results are in harmony with Way's data on pipe clay and finely ground burnt clay pipes. They show that these materials contain substantial amounts of bases in replaceable form which are not necessarily converted into non-replaceable forms either by the consolidating processes involved in the formation of shale or by relatively high temperature.

A light-colored, slightly compacted, shale-like layer about one inch in thickness occurs about two feet below the surface where soil 539 was drawn. This material was free from  $\text{CaCO}_3$  but contained 1.58 per cent replaceable Ca. Gedroiz reported several clay soils from Russia with more than one per cent of replaceable Ca.

As stated above the replaceable bases of normal non-acid soils are composed mainly of Ca, with Mg present in considerable amount and with quite low percentages of monovalent bases. This fact was explained by Gedroiz on the basis of relative solubilities. During the process of formation and subsequently, the most soluble constituents would, of course, be most likely to pass out into the drainage. Another factor is found in the fact that insoluble  $\text{CaCO}_3$  is frequently laid down along with the other constituents. Regardless of the origin of the replaceable bases, the relative proportions of the different bases originally present, or whether they are components of definite chemical compounds or are adsorptively held, the gradual solution of  $\text{CaCO}_3$  will result in the substitution of Ca for the other replaceable bases. Since the monovalent bases are somewhat more easily replaced than Mg, the tendency is towards a low percentage of monovalent bases and a high percentage of Ca, with Mg somewhat intermediate.

Hissink has pointed out that this process has actually taken place to a demonstrable extent in certain polder soils of Holland. These soils soon after having been flooded by sea water less than a century ago contained considerably less replaceable Ca and correspondingly more Na than at present, but they have sustained considerable loss of  $\text{CaCO}_3$  since these areas were first reclaimed. It seems probable that  $\text{CaCO}_3$ , brought into solution as the bicarbonate, has displaced Na from its position in the replaceable complex. We believe that similar reactions may be promoted in the reclamation of alkali soils, with the consequent improvement of such soils.

#### EFFECT OF DILUTE ACIDS

Gedroiz<sup>18</sup> claims that when a soil is treated with dilute acid, the bases are replaced by hydrogen. We have made a brief study of this point, using water saturated with  $\text{CO}_2$  and also three strengths of HCl. Twenty-five-gram portions of soil 431 were shaken with the solutions at room temperature and leached with the solutions until one liter of

the HCl extract and four liters of the carbonic-acid extract were obtained. Each liter of the latter was analyzed separately. It was found that the first liter contained much the greatest amount of Ca, but the last also contained appreciable amounts. The total amounts of bases dissolved are recorded in table 12.

TABLE 12  
EFFECTS OF DILUTE ACIDS AS COMPARED WITH  $\text{NH}_4\text{Cl}$

Solution used	Per cent of soil							
	Ca	Mg	K	Na	Al	Fe	Mn	$\text{SiO}_2$
$\text{H}_2\text{O}$ saturated with $\text{CO}_2$	0.105	0.030	0.014	**				0.089
0.01N HCl.....	.283	.040	.031	.017	.081		.003	.060
0.02N HCl.....	.336	.052	.033	.026	.103		.015	.081
0.04N HCl.....	.440	.070	.055	.031	.152*	**	**	.140
1.0N $\text{NH}_4\text{Cl}$ .....	.436	.073	.053	.028				.054

\* Precipitate contained some iron.

\*\* Not determined.

As the strength of the acid was increased the amount of bases dissolved increased. With the 0.04N HCl almost exactly the same amounts of Ca, Mg, K and Na were found as were replaced from the original soil by normal  $\text{NH}_4\text{Cl}$ , but considerable Al, Fe and Mn were dissolved also.

The soil residues from the preceding experiment were leached with water until free from Cl. They were then extracted with normal  $\text{NH}_4\text{Cl}$  exactly as in the preceding replacement studies. The results recorded in table 13 show that the soil previously extracted with dilute acid still contained considerable amounts of replaceable bases. As stated already this soil originally contained only the merest traces of trivalent bases soluble in  $\text{NH}_4\text{Cl}$ . Treatment with dilute HCl so affected the soil, however, as to render the Al, Fe and Mn distinctly replaceable by  $\text{NH}_4\text{Cl}$ . Carbonic acid on the other hand had no effect on the replaceability of these constituents. The solubility of  $\text{SiO}_2$  was also materially increased. The amount of  $\text{NH}_4$  absorbed from  $\text{NH}_4\text{Cl}$  was reduced somewhat but not greatly by the preliminary treatment with acid (compare tables 5 and 13).

TABLE 13  
CONSTITUENTS DISSOLVED BY NH<sub>4</sub>Cl AFTER EXTRACTION WITH DILUTE ACID

Soil previously extracted with	Per cent of soil							Milligram equivalents							NH <sub>4</sub> ab- sorbed		
	Ca	Mg	K	Na	Al	Fe	Mn	SiO <sub>2</sub>	Ca	Mg	K	Na	Al	Fe		Mn	Total bases
H <sub>2</sub> CO <sub>3</sub> .....	.342	0.058	0.028	.016	.....	.....	.....	*	17.1	4.8	0.7	0.7	.....	.....	.....	23.3	25.4
0.01N HCl.....	.197	.011	.019	.014	.057	.007	.005	.134	9.8	9	.5	.6	6.3	.4	.3	18.8	23.3
0.02N HCl.....	.161	.017	.024	.014	.074	.012	.009	.161	8.0	1.4	.6	.6	8.2	.7	.5	20.0	22.8
0.04N HCl.....	.081	.051	.029	.036	.088	.047	*	.177	4.0	4.2	.7	1.6	9.8	2.6	.....	22.9	23.9

\* Not determined.

If treatment with dilute acids merely brings about a substitution of hydrogen for the several bases present in replaceable form, then the amount of the bases dissolved by the acid plus that replaced by  $\text{NH}_4\text{Cl}$  after the acid treatment should approximate the amounts of replaceable bases originally present. The data recorded in table 14 show that this was the case when carbonic acid was used, but not so with dilute  $\text{HCl}$ . These determinations indicate that dilute  $\text{HCl}$  attacks not only the constituents normally involved in replacement reactions but still others as well. When the strength of the acid is sufficient to dissolve quantities of bases similar to those which are replaceable by neutral salt solutions, it seems that rather deep-seated chemical reactions take place which are not involved in replacement with salts.

TABLE 14

TOTAL AMOUNTS OF CONSTITUENTS EXTRACTED BY ACIDS AND  $\text{NH}_4\text{Cl}$ 

	Per cent of soil							
	Ca	Mg	K	Na	Al	Fe	Mn	$\text{SiO}_2$
$\text{NH}_4\text{Cl}$ alone.....	0.436	0.073	0.053	0.028	.....	.....	.....	0.054
$\text{H}_2\text{CO}_3$ and $\text{NH}_4\text{Cl}$ .....	.447	.088	.042	*	.....	.....	.....	.089
0.01N $\text{HCl}$ and $\text{NH}_4\text{Cl}$ ..	.480	.051	.050	.031	.138	.007	.008	.194
0.02N $\text{HCl}$ and $\text{NH}_4\text{Cl}$ ..	.497	.069	.057	.040	.177	.012	.024	.242
0.04N $\text{HCl}$ and $\text{NH}_4\text{Cl}$ ..	.521	.121	.084	.067	.240	.047	*	.317

\* Not determined.

It is difficult to say whether the trivalent bases reported in table 13 were really brought into solution as a result of replacement or by solubility effects. The fact that the total amount of bases found, including Al, Fe and Mn, when expressed as chemical equivalents, was only slightly less than the  $\text{NH}_4$  absorbed suggests that these bases were actually replaced, but it is possible, although hardly probable, that  $\text{NH}_4$  replaced hydrogen thus producing a solution sufficiently acid to effect the solution of these bases. A thorough understanding of the reactions which take place when soils are treated with dilute acids might throw important light on the origin of replaceable Al, Fe and Mn in acid soils.\*

\* After this paper had gone to press our attention was called to the investigations of Daikuhara (Bul. Imp. Cent. Agr. Exp. Sta., Japan, 2: 1-40, 1914) and Liesegang and Kappen (Landw. Vers. Sta., 99: 191-230, 1921) in which the subject of soil acidity was discussed from the standpoint of replacement. The latter showed that the acidity of  $\text{KCl}$  extracts of certain naturally acid soils and of soils that had been treated with dilute acids was due entirely to replaceable Al. The mechanism by which Al becomes replaceable upon treating a neutral soil with dilute acids was also discussed.



Extraction with dilute HCl, however, does not necessarily affect the capacity of the soil to hold replaceable bases as is shown by the following experiment. The same soil was first extracted with 0.02N HCl, then leached free from Cl. The residue was treated with  $\text{Ca}(\text{OH})_2$  solution and again leached with water until practically free from soluble Ca. The soil was then treated with normal  $\text{NH}_4\text{Cl}$  solution as in the preceding determination of replaceable bases. It was found that the total content of replaceable bases as measured by the  $\text{NH}_4$  absorbed was thus restored to its original amount.

The above data suggest that certain soils are acidic not merely because they contain replaceable hydrogen in the sense employed in this paper but because of the presence of soluble trivalent bases which form hydrolyzable salts or possibly because of the presence of silicic acids. The application of lime to such soil would be expected to lower the solubility of these bases and at the same time augment the supply of Ca that is readily soluble in carbonated water.

It is not possible at present to explain the fact that soils of similar type vary so greatly in replaceable base content. There is some evidence that, other things being equal, the extent to which the soil materials have been weathered and the amount of leaching the soil has undergone determine the content of replaceable bases. Meteoric waters undoubtedly tend to displace these bases but this would hardly lower the total content very materially as long as  $\text{CaCO}_3$  is present. When the carbonate has been exhausted the replaceable bases probably gradually disappear. It is well known that  $\text{CaCO}_3$  frequently occurs in considerable amount in the substratum a foot or more below the surface, while the surface soil may be free from  $\text{CaCO}_3$ . This is especially true in semi-arid regions and often where there is no evidence that  $\text{CaCO}_3$  was an original constituent of the soil. It is probable that the carbonate has been derived from the replaceable Ca.

#### ADSORPTION

Our results agree with those of Gedrioz and Hissink in showing that the rate at which the replacement takes place is extremely rapid. Hissink holds that this is evidence that the replaceable bases occur on the surface of the soil particles. Since the reaction between salt solutions and soils involves an interchange of bases with little or no change in the anions of the soil or the solution, Hissink believes, in



common with many other workers, that the bases are present in a state of adsorption. Much of the published literature on this subject is characterized, however, by vagueness. Frequently there is but little indication as to the meaning intended to be conveyed by the term adsorption. It seems to have been employed by various writers on soils more as a convenient word than to express a definite idea.

Hissink hypothesizes that certain anions, possibly  $\text{SiO}_3$  and organic radicles, and the cations involved in replacement form a sort of electric double layer around the soil particles. The anions bearing a negative charge are considered to be on the interior and the cations with their positive charge on the exterior of this double layer. Hence an exchange of cations takes place between the soil and the solution without any effect on the anions. This view suggests the well known electric-double-layer theory of Helmholtz and the applications that have been made of it by various students of cataphoresis and adsorption phenomena. This view differs from the Helmholtz theory, however, in that the double layer is regarded by Hissink as an essential part of the soil particles themselves, whereas in the application of the Helmholtz theory to other systems the disperse phase and the dispersion medium are both usually considered as contributing to the formation of the electric double layer. This latter assumption seems to be necessary to account for the fact that certain colloids are electro-negative when dispersed in one medium and electro-positive in another medium.

Although it is true that various crystalline silicates are capable of undergoing replacement reactions as shown by Lemberg,<sup>25</sup> Sullivan,<sup>34</sup> Cummins and Kelley<sup>7</sup> and others, it seems safe to say that with the soils we have studied and possibly with soils generally the bases have become replaceable mainly as a result of metamorphosis occasioned by the weathering process. Whether the bases occur in ordinary chemical union with silica as aluminosilicates, or are held in a state of adsorption, can not now be definitely stated.

As shown in the preceding section of this paper, soils both acid and neutral have the power to absorb substantial amounts of  $\text{Ca}(\text{OH})_2$  without exchange of cations. When thus absorbed  $\text{Ca}(\text{OH})_2$  appears to be loosely held. It slowly passes into solution with water, yielding an alkaline solution. Replaceable Ca, on the other hand, is practically insoluble in water. Mattson<sup>27</sup> explained the fact that clay and certain other colloids absorb  $\text{Ca}(\text{OH})_2$  on the assumption that these materials

first take up the OH ions by adsorption. This increases the negative charge of the particles and promotes the adsorption of the positively charged Ca ions. Vernadsky<sup>40</sup> has recently pointed out that numerous aluminosilicates, including the zeolites, feldspars, etc., have a common aluminosilicate nucleus, and that these silicates form a series of addition compounds with  $\text{Ca}(\text{OH})_2$ . He also claims that other compounds of aluminum and silicon, not belonging to the aluminosilicate class, also form addition compounds with  $\text{Ca}(\text{OH})_2$ .

The results of recent investigations in physical chemistry indicate that there is no sharp distinction between ordinary chemical union and adsorption processes. Some prominent workers in this field look upon the process of adsorption which finely divided materials, clay in particular, manifest in striking degree as being essentially chemical. Langmuir<sup>24</sup> presented evidence which supports this view. He considers the forces involved in adsorption to be analogous to those taking part in the union of molecules in crystal formation. X-ray examination of various crystals has indicated that the atoms are not combined as in the gaseous form of the compound. The ordinary valency appears to be supplanted by what has been designated as residual or secondary valency. Recently Svedberg<sup>35</sup> has stated that Langmuir's theory offers the best explanation yet advanced for the adsorption of ions by colloids. The formation of the addition compounds of silicates and  $\text{Ca}(\text{OH})_2$  just referred to and the absorption of  $\text{Ca}(\text{OH})_2$  by soils might be similarly explained.

G. N. Lewis<sup>26</sup> has undertaken to show the mechanism of this process on the basis of the electronic concept. In his view the ordinary chemical bond is a pair of electrons shared mutually by two atoms. He suggests that crystals are built up by the sharing of pairs of electrons which occur on the surface of molecules and that adsorption implies the sharing of previously unshared pairs of electrons between the substances involved. He points out that the well known adsorptive power of  $\text{SiO}_2$  is in harmony with this theory. Still others claim that adsorption of ions or compounds from solutions involves hydrolysis. Quite recently Miller<sup>27</sup> has shown that carbon absorbs bases from solutions of electrolytes as a result of hydrolysis.

The tendency, therefore, is to bring adsorptive processes into line with chemical principles.

There is much evidence that the taking up of  $\text{Na}_2\text{CO}_3$  and soluble phosphates by soils and the absorption of dyes,<sup>12</sup> which have long been explained on the basis of adsorption, are essentially chemical processes involving the formation of insoluble compounds. For example, soil 431 has the power to absorb notable amounts of  $\text{Na}_2\text{CO}_3$ , but after the replaceable Ca and Mg have been substituted by Na this soil no longer has this power. Bradfield<sup>3</sup> has shown that the action of dilute alkali on colloidal clay obeys definite chemical laws. The reaction is in harmony with the assumption that the colloid contains a weak acid of low solubility. In his opinion it is unnecessary to take recourse to adsorption concepts in order to explain soil acidity, as has been done by numerous writers on the subject. Truog,<sup>36</sup> Sharp and Hoagland<sup>33</sup> and others hold similar views.

The experimental data presented above indicate that the replaceable bases are chemically combined in soils. Some recent data suggest that the content of replaceable bases is one of the fundamental characteristics of soils. While much still remains to be determined, we believe that the replaceable bases occur as chemical compounds, probably as complex alumino silicates which have been formed as a result of weathering. There seems no good reason why such compounds may not be formed, and certainly the assumption of such vaguely defined forces as are commonly understood by the term adsorption does not help to clarify a subject which by its very nature is extremely complex.

From this point of view these constituents may be looked upon as being similar to the zeolites, as has long been held to be the case. They represent a transition stage between the igneous minerals and insoluble oxides or kaolin but can not be identified petrographically because of the small size of the particles. Strong support for this view is afforded by the mode of occurrence and formation of the crystalline zeolites in the state of nature. The reactive constituents of soils are not necessarily zeolitic, however, for certain nonzeolitic minerals undergo basic exchange with salt solutions.

Recently Ganssen (Gans)<sup>10</sup> has discussed the composition of the zeolitic constituents of soils on the basis of the ratio of  $\text{SiO}_2$ :  $\text{Al}_2\text{O}_3$ : bases. The analyses upon which his discussion rests were made by acid digestion. Gedroiz<sup>13</sup> has shown that acids of the strengths used in making these analyses dissolve substantial amounts of bases which do not undergo exchange reactions. Moreover, no method has yet been

discovered by which the complex which is involved in the replacement phenomenon can be decomposed into its components without decomposing other constituents as well. By separating the colloidal material from the soil by mechanical means and then studying its composition and properties, important light may be thrown on the composition of the constituents involved in replacement reactions.

As stated before the replacement reactions appear to take place almost instantaneously. They do not go to completion at equal rates throughout, however. Gedroiz has repeatedly emphasized that it requires prolonged extraction in order to effect the replacement of the last traces of Ca, but that the replaceable Mg, K and Na may be more readily displaced. He was unable to effect complete replacement with 0.2 normal solutions of salts even when the soluble products were removed by repeated decantation. However, partial replacement took place at once with this and even less concentrated solutions. Hissink also found that a lesser amount of leaching with salt solutions is required to remove the replaceable monovalent bases than the divalent bases. Our experience is in agreement with theirs. It is difficult to harmonize these facts with the adsorption hypothesis of Hissink. If every atom of a given base is located on the exposed surface of particles, as he assumes, one should be as readily displaced as another.

The fact that a part of the replaceable Ca, and possibly of other bases as well, is more easily replaced than other parts suggests that more than one chemical compound is involved. Considering the great variation in the composition of natural silicates, including the true zeolites, the probable variation in the structural arrangement within the various molecules of silicates, and the numerous possibilities for chemical combinations between the several silicic acids and the different bases, it is almost certain that a varied assortment of degradation products is formed during the course of weathering. The differences in the rate of replacement might also be due in part at least to the occurrence of molecular aggregates of the replaceable compounds, sub-microscopic crystals in fact, some of whose chemically combined bases occur on the interior of the particle and can be replaced only as a result of diffusion.

It should not be inferred from the preceding discussion that organic substances are not involved in the phenomenon of replacement. On the contrary there is much evidence that a part of the replaceable



bases usually occurs in some sort of combination with organic matter. These constituents probably exert important influences on soils. It has seemed logical in our treatment of this subject, however, to limit the present discussion to the inorganic constituents.

### SUMMARY

1. By taking advantage of the exchange reaction which takes place between soils and  $\text{NH}_4\text{Cl}$  the total content of the several bases present in replaceable form may be readily determined. In order to effect complete replacement it is necessary either to extract a given sample of soil many times with the salt solution, or else subject it to leaching conditions. The latter is the more practicable.

2. The exchange of bases proceeds to completion only when a sufficiently concentrated solution is used. With  $\text{NH}_4\text{Cl}$  this concentration is approximately normal. Since the exchange takes place stoichiometrically, the amount of  $\text{NH}_4$  absorbed by the soil is chemically equivalent to the sum of all the bases replaced. The absorbed  $\text{NH}_4$  may be determined by first leaching out the excess of  $\text{NH}_4\text{Cl}$  and then distilling the residue with a solution of  $\text{NaOH}$  or some other alkaline solution of sufficient concentration.

3. The difference between the absorbed  $\text{NH}_4$  and the sum of the several bases extracted represents solubility effects rather than replacement. This difference is not great except with soils which contain considerable amounts of soluble salts or insoluble carbonates. The amount of  $\text{CaCO}_3$  dissolved by  $\text{NH}_4\text{Cl}$  may be calculated from  $\text{CO}_2$  determinations made before and after the extraction.

4. The replaceable bases of several neutral or slightly alkaline soils from California are composed mainly of Ca (50 or more per cent of the total replaceable bases); Mg is present in the next highest amount (20 to 30 per cent), but there are only small amounts of K and Na.

5. Alkali soils are characterized by a relatively large amount of replaceable Na and a correspondingly low amount of replaceable Ca. Several black-alkali soils were studied which contain no replaceable Ca. The relative relationship between the several replaceable bases of alkali soils depends on the composition and concentration of the soluble salts. Where the soluble salts contain sufficient amounts of Ca compounds, the ratio of the replaceable bases may be similar to that of

normal soils. Replaceable Na must be reckoned with in the practical treatment of alkali soils.

6. The acid soils examined are characterized by a low total content of replaceable bases, and by the presence in replaceable form of more or less trivalent bases (Al, Fe or Mn). The amount of these latter bases may constitute 50 or more per cent of the total replaceable bases.

7. Acid soils have the power to absorb substantial amounts of  $\text{Ca}(\text{OH})_2$ . A part of the absorbed  $\text{Ca}(\text{OH})_2$  may enter into replaceable form, but a much larger part may not. The absorption of  $\text{Ca}(\text{OH})_2$  is not confined to acid soils, however. A neutral soil was studied which absorbed large amounts of  $\text{Ca}(\text{OH})_2$  without an exchange of cations.

8. The greater part of the replaceable bases reside in the clay fraction of soils, but different clays vary greatly in their total content.

9. The hydrogen of carbonic acid may be substituted for a part of the replaceable bases. Dilute HCl likewise displaces the bases, but may attack other constituents as well. A neutral soil, which originally contained mere traces of trivalent bases in replaceable form, was found to contain substantial amounts after treatment with dilute HCl.

10. The replaceable bases are considered to be present not in a state of physical adsorption but as chemical compounds, probably as complex alumino silicates which have been formed as a result of weathering.

11. The results obtained in this investigation are in close agreement with those published by Gedroiz and Hissink.



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THE MOISTURE EQUIVALENT AS INFLUENCED  
BY THE AMOUNT OF SOIL USED  
IN ITS DETERMINATION

BY

F. J. VEIHMAYER, O. W. ISRAELSEN AND J. P. CONRAD

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Contribution from the Division of Irrigation Investigations and Practice\*  
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\* The work on which this paper is based has been done in the laboratory of the Division of Irrigation Investigations and Practice, with O. W. Israelsen (of the Department of Irrigation and Drainage, Utah Agricultural College), graduate student in soil physics and irrigation, and J. P. Conrad, of the Division of Agronomy, collaborating. Some financial assistance was rendered by the Division of Agricultural Engineering, Bureau of Public Roads, U. S. Department of Agriculture.

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## INTRODUCTION

Moisture equivalent and hygroscopic determinations constitute the more important quantitative bases of comparisons of the moisture properties of soils. The mechanical analysis, while furnishing important data for interpreting the physical properties of soils, can scarcely be used to compare moisture retentiveness. The hygroscopic coefficient determination made in accordance with Hilgard's<sup>7, 8</sup> method presents certain difficulties, as pointed out by Lipman and Sharp<sup>10</sup> and Alway and Russell.<sup>1</sup> The method requires provision for the maintenance of a constant temperature in the room in which the soils are exposed and also the insurance of the actual saturation of the atmosphere, conditions usually difficult to secure. The moisture equivalent can be determined with much greater facility, requiring only a simple piece of apparatus which is easily operated. Further, since it furnishes a single-valued moisture constant, it has been widely used in interpreting soil moisture studies. The determination of a relationship between the moisture equivalent and other physical properties of soils, together with its value as an indirect measure of the wilting coefficient, has still further widened its use. Thus the moisture equivalent has come to be as much the tool of the ecologist and plant physiologist as it is of the soil physicist and irrigationist.

Consideration of the accuracy and reliability of moisture equivalent determinations, therefore, is very important. In making moisture equivalent determinations in this laboratory to be used as a basis of comparison between soils, inconsistencies were often observed. Repeated trials on the same soil have sometimes given appreciable

variations in the values obtained. These variations often were of such magnitude that they could not be attributed to soil differences. Trials made by one of us<sup>13</sup> with samples of varying amounts of the same soil indicated that the value obtained for the moisture equivalent is materially influenced by the amount of soil used in its determination. However, it has been found that variations occur in different trials with the same soil, even though the amount of soil taken is the same. This is due to causes, the extent and magnitude of which are not yet ascertained.

The moisture equivalent as a soil moisture constant was first proposed by Briggs and McLane<sup>3</sup> in 1907. It occurred to these authors that a satisfactory basis for comparing the moisture-holding capacities of soils might be established by determining the amount of moisture which different soils are capable of retaining when that moisture is subjected to a constant measured force, sufficient in magnitude to remove the water held in the larger capillary spaces. They define the moisture equivalent as "the percentage of water retained by a soil, when the moisture content is reduced by means of a constant centrifugal force until it is brought into a state of capillary equilibrium with the applied force."

In their first publication they described the method and apparatus to be used. The effects of duration of test, of initial water content and of speed, on the moisture equivalent, were investigated. No definite procedure, however, was suggested. The results with over one hundred samples were determined by the use of a centrifugal force of 3000 times gravity. The duration of the tests was thirty minutes. These authors suggested that it might be desirable to make the tests at a speed such that the soil would be subjected to a centrifugal force of 1000 times gravity. No information on the weight of soil used in the determination was given other than to state that "the amount of soil used covered the bottom of the cylindrical cup (internal diameter 3.7 cm.) to a depth of approximately 5 mm." The statement was also made that when larger amounts of soil were used there was a tendency for a part of the water to accumulate upon the top of the soil.

In 1910, Briggs and McLane<sup>4</sup> described a modified apparatus for determining the moisture equivalent, and recommended the use of a centrifugal force equal to 1000 times gravity. The samples were centrifuged for a period of forty minutes, which the authors found

sufficient to establish a condition of practical equilibrium. It was suggested that the soil to be examined be put through a 2-millimeter sieve and then placed in the centrifuge cups, the wire gauze bottom of each cup first being covered with a sheet of filter paper. An amount of soil was taken sufficient to give a packed layer of one centimeter in thickness. It is stated that usually this will be about 30 grams, the amount by volume being determined by filling a measuring cup level full. The cups of soil should be "thoroughly moistened (not saturated) without stirring, and allowed to stand protected from evaporation for about 24 hours."

The method above, recommended by Briggs and McLane, has come to be the procedure followed in many laboratories in making moisture equivalent determinations, except that usually the soil is centrifuged for a period of thirty minutes after the centrifuge has reached the desired speed.

#### VARIATIONS IN MOISTURE EQUIVALENT DETERMINATIONS PREVIOUSLY NOTED

Before proceeding with the presentation of the experimental data, it seems advisable to consider briefly some of the factors which may influence the determination of the moisture equivalent, and also to call attention to some publications which refer to observed variations in such determinations.

Briggs and McLane<sup>4</sup> point out that since the surface tension of water decreases as the temperature increases at the rate of about 0.2 per cent per degree Centigrade, the moisture equivalent determination is dependent to a certain extent upon temperature. They adopted 20° C. as the standard temperature, but state that the temperature effects can usually be disregarded. The effect of temperature precludes the possibility that the variations which we have observed were due to this cause, if surface tension is the only factor involved in temperature changes.

Briggs and McLane<sup>3</sup> determined the effect of speed. Their data show that the centrifugal force must be appreciably increased to change the moisture equivalent materially. It is apparent from their results that the variations in moisture equivalent which we observed cannot be attributed to the accidental departure from the desired speed of 2440 r.p.m. which may have taken place during the tests.



Briggs and McLane<sup>4</sup> further report a series of moisture equivalent determinations made upon a certain soil on one day, and a second series made on another day, using samples about one half the size of those of the first series. The larger samples showed an average moisture equivalent of 18.48 per cent, the smaller samples of 18.45 per cent. These authors thought this small variation was due in part to errors arising in connection with the moisture determinations of small samples. They clearly did not consider the size of the sample as exerting an influence on the moisture equivalent.

Thomas<sup>12</sup> determined the moisture equivalents of a loam soil when varying amounts of soil were placed in the centrifuge cups. He showed that the moisture equivalent decreases as the thickness of the soil layer in the centrifuge cups increases. This decrease is attributed by him to the counter-balancing effect of a negative moisture gradient.

Joseph and Martin<sup>9</sup> report the moisture equivalents of several soils and point out that the moisture equivalent diminishes with increasing quantity of soil taken for the sample. They believe this to be due to the packing of the outer layers of soil in the centrifuge cups. They also point out that with some heavy soils the moisture equivalent will increase, instead of diminish, when the amount of soil is increased. This is due to the accumulation of water on top of the soil because of the increase in density of packing and consequent impermeability.

The data embodied in the present paper are based principally upon determinations made on five different soils, descriptions of which follow.

#### DESCRIPTION OF SOILS USED

All of the stock supplies of the different soils used were collected in areas free from weed growth, and were in an air-dried condition. The samples were collected at different times, and consequently some of them were stored for much longer periods than others before they were centrifuged. For instance, the Yolo clay loam, from the Santa Clara Valley of California, upon which the preliminary work was done, was the first sample collected. It was stored in the laboratory for over two years before some of the other soils were collected.

A sample of about 75 pounds was taken in each instance. The soil was carefully screened and thoroughly mixed a number of times by heaping and turning. The samples were passed through a 2-mm.

sieve before being used for moisture equivalent determinations. The manner of selecting and preparing the samples insures, as nearly as possible, the use of a uniform soil for the different determinations of each type.

The soils used, the places from which they were collected, their published descriptions, and their specific gravities are tabulated below.

Soil	Specific gravity	Place collected	Publication containing description of type of soil
Yolo clay.....	2.724	In the vicinity of Davis, California.	Reconnaissance Soil Survey, Sacramento Valley, California. U. S. Department of Agriculture, Bureau of Soils, 1913.
Yolo clay loam.....	2.685	Deciduous Fruit Station of the University of California at Mountain View, in the Santa Clara Valley, California.	Reconnaissance Soil Survey of San Francisco Bay Region, California. U. S. Department of Agriculture, Bureau of Soils, 1914.
Yolo silt loam.....	2.718	Agronomy plots, University Farm, Davis, California.	Reconnaissance Soil Survey, Sacramento Valley, California. U. S. Department of Agriculture, Bureau of Soils, 1913.
Twin Falls silt loam.....	2.705	Twin Falls, Idaho.....	Tentatively classed as Twin Falls silt loam in Reconnaissance Soil Survey which will shortly be published by Bureau of Soils.
Oakley fine sand....	2.663	Experimental Irrigation tract on State Land Settlement Colony at Delhi, California.	Described as Oakley sand undifferentiated in Reconnaissance Soil Survey, Lower San Joaquin Valley, California, Bureau of Soils, 1915.

The Yolo clay loam from the Santa Clara Valley, upon which most of the work reported in this paper was done, is lighter in texture, and is nearer than its name would signify, to the silty clay loam series described in the Reconnaissance Soil Survey of the San Francisco Bay Region Advance Sheets—Field Operations of the Bureau of Soils, 1914, page 88. The real specific gravities of the soils given in the tabulation are the averages of closely agreeing duplicate determinations in each case.



## SECTION I

## INFLUENCE OF THE WEIGHT OF SOIL USED

In the work reported below, the standard moisture equivalent centrifuge was used. This was manufactured by the International Equipment Company and is of the type described by Briggs and McLane,<sup>4</sup> except that the centrifugal head is enclosed in a metal case provided with a hinged cover. With the exception of certain extra precautions, the procedure in making the moisture equivalent determinations was in accordance with the usual practices in most laboratories. The centrifuge cups, after being fitted with accurately cut squares of filter paper, were filled with the samples to be centrifuged. The surface of the soil was carefully leveled by means of hard wood blocks, 4.7 cm. square and about 6 cm. long, whose bottoms were cut to a radius of about 15 cm. (the same radius as that of the curve of the centrifuge cups). After the soil surfaces in the centrifuge cups were smoothed and leveled off with these blocks, they were leveled approximately with a putty knife. Just enough pressure was applied with the block to settle the soil particles lightly in place. Care was taken not to compact the soil to a greater degree than would result from the centrifugal force. An ordinary tin cake pan, 8½ inches square and 1 inch deep, was used for storing and wetting the samples, as such a tin holds the 16 centrifuge cups conveniently (pl. 2, fig. 1). A series of holes was drilled in the bottom of the pan; the bottom was then covered with one or two layers of toweling upon which was placed a layer of filter paper. The centrifuge cups were then placed in the cake pan, and distilled water carefully poured onto the filter paper, a sufficient quantity of water being applied to cause moisture to appear on the surface of the soil in the cups. The pan, with the cups, was then placed in another unperforated pan of the same size and the whole covered until ready for use. The samples were allowed to stand for twenty-four hours after wetting, except as otherwise noted.

In every case, except where indicated, the duration of the test was thirty minutes after the centrifuge had been brought up to the required speed. The speed of the centrifuge was controlled by hand manipulation of the rheostat, since it was found that the Kellogg governor could not be depended upon to give the desired regulation.

TABLE 1  
THE PERCENTAGE OF MOISTURE RETAINED AFTER CENTRIFUGING BY SAMPLES OF SOILS OF DIFFERENT WEIGHTS

Soil	Number of determinations for each size sample	Weights of samples of air-dried soil, gms.								For illustration see—
		5	10	15	20	30	40	50	60	
Yolo clay	4		36.4 ±0.12		32.6 ±0.18	30.1	31.0 ±0.16	39.2 ±0.32	37.8 ±0.21	Fig. 1, curve 1, and fig. 2.
Yolo clay loam*	16	46.1 ±0.19	38.2 ±0.12		30.1 ±0.04	26.9 ±0.05	25.1 ±0.08	24.1 ±0.02	23.5 ±0.02	
Yolo clay loam	8		31.2 ±0.23		26.7 ±0.08	23.9 ±0.22	22.4 ±0.09	20.0		
Yolo silt loam	8	36.2 ±0.08	30.9 ±0.30		27.2 ±0.14	26.0 ±0.07	25.0 ±0.03	24.1 ±0.08	23.6 ±0.04	Fig. 1, curve 2. Fig. 1, curve 3.
Yolo fine sandy loam*	8		34.5 ±0.15	36.6 ±0.07		24.6 ±0.03	23.0 ±0.04			Fig. 1, curve 4. Fig. 1, curve 6.
Twin Falls silt loam*	8		34.5† ±0.75	31.3 ±0.08		25.2 ±0.06	22.0† ±0.13			
Twin Falls silt loam	8		28.6 ±0.13		24.9 ±0.07	22.5 ±0.05	21.3 ±0.05	20.3 ±0.09	19.8 ±0.05	
Oakley fine sand*	8		14.2 ±0.11	10.8 ±0.06		7.9 ±0.03	7.2 ±0.10			Fig. 1, curve 5.
Oakley fine sand	8		8.6† ±0.10		6.7 ±0.05	5.8 ±0.05	5.4 ±0.06	5.1 ±0.05	4.8 ±0.05	

\* These samples were dried in centrifuge cups and therefore include the moisture in the filter paper.

† Only four determinations were made in this case.

The speed of the centrifuge was indicated by a Frahm speed indicator and was maintained in every case between 2425 and 2450 revolutions per minute.

The moisture determinations were made by drying the whole sample. In some instances, as noted, the samples were not removed from the centrifuge cups after being centrifuged, but were placed directly in weighing cans and the moisture determinations made in the usual manner. In the majority of cases, however, the usual practice of removing the sample from the cups and of discarding the filter paper was followed. In the former case the moisture equivalent determination resulted in a higher value than in the latter, since the water retained by the filter paper in opposition to the centrifugal force and to the compression due to the layer of soil is counted as loss of moisture on drying. This phase of the problem will be dealt with later in this paper. All of the moisture determinations were made by drying the samples in a thermostatically-controlled electric oven. The drying temperature was maintained between 110° and 115° C. The samples were repeatedly check-weighed before the final dry weights were recorded. The moisture equivalents were obtained by using samples of varying amounts of six different soils. The samples used were weighed quantities of air-dried soil. The data obtained from the determinations are presented in table 1 and graphically illustrated in figure 1. In table 2 are listed the weights of oven-dried soil corresponding to the air-dried samples.

TABLE 2  
AVERAGE WEIGHT IN GRAMS OF OVEN-DRIED SOIL FOUND IN DETERMINING  
PERCENTAGE OF MOISTURE RETAINED REPORTED IN TABLE 1

Soil	Number of determinations	Weights of samples of air-dried soil, in grams.							
		5	10	15	20	30	40	50	60
Yolo clay.....	4		9.5		19.1	28.6	38.0	47.1	56.6
Yolo clay loam*....	16	4.7	9.6		19.3	29.0	38.5	48.2	57.6
Yolo clay loam.....	8		9.7		19.4	29.2	39.0	48.6	
Yolo silt loam.....	8	4.8	9.5		19.1	28.7	38.3	47.8	57.3
Yolo fine sandy loa m*	8		9.6	14.8		29.6	39.0		
Twin Falls silt loam *	8		9.6	14.1		28.4	38.8		
Twin Falls silt loam	8		9.6		19.3	29.0	38.7	48.4	58.1
Oakley fine sand*..	8		9.8	14.8		29.7	39.7		
Oakley fine sand....	8		9.8		19.8	29.8	39.8	49.7	59.7

\* These samples were dried in centrifuged cups, but the weight of soil does not include the weight of filter paper.

It was noted that in all cases, except those of the 15-gm. samples of the Yolo fine sandy loam and of the 40, 50, and 60-gm. samples of the Yolo clay, the percentage of moisture retained decreased when the amount of soil used in making the determination was increased. The reason for the discrepancy in the case of the 15-gm. sample of Yolo fine sandy loam is not now apparent.

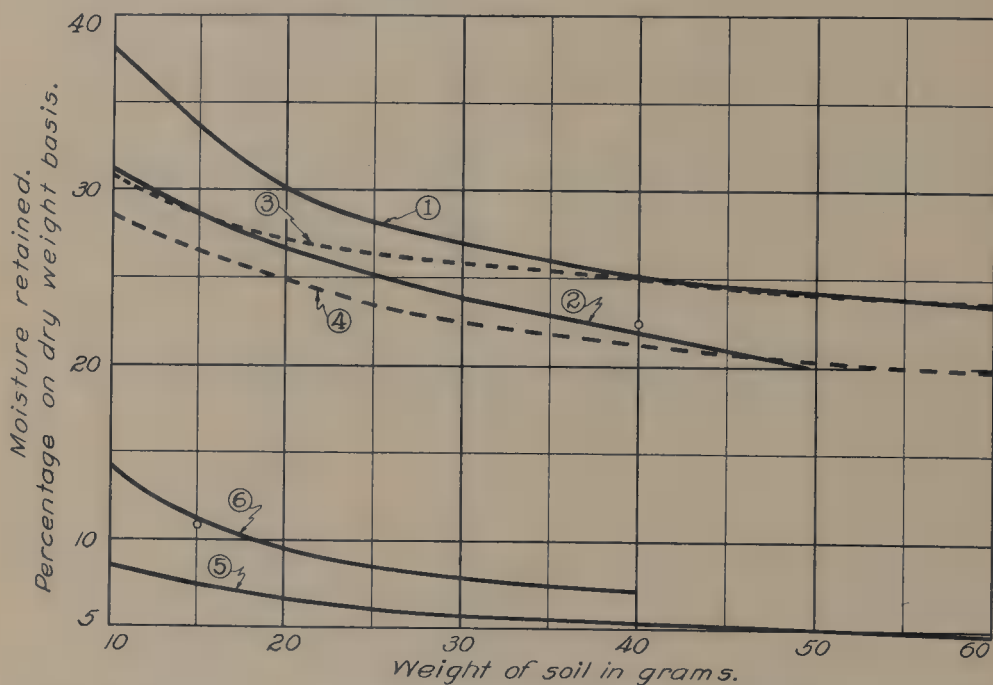


Fig. 1. Percentage of moisture retained against the centrifugal force of 1000 times gravity as determined by the use of different weights of soil in the centrifuge cups. Curves 1 and 2, Yolo clay loam; curve 3, Yolo silt loam; curve 4, Twin Falls silt loam; curves 5 and 6, Oakley fine sand.

This discrepancy may be accounted for by the fact that these determinations were made at different times. As has been pointed out, repeated trials made on the same soil at different times have shown variations in the results. This variation in values is clearly shown by a comparison of the two sets of data in table 1 for the Yolo clay loam, the Twin Falls silt loam, and the Oakley fine sand; it is also evident in the curves of figure 1. The determinations of these particular soils were made at various times extending over a period of more than two years. In the case of each of these three soils, the determinations of the first set of samples tested, which were dried in the centrifuge cups, were made in a single run of the centrifuge. Sixteen cups were filled with samples of uniform size from Yolo clay loam and were centrifuged simultaneously.

In the case of the Yolo fine sandy loam, the Twin Falls silt loam, and the Oakley fine sand, eight cups of one-size sample and eight of another were centrifuged together. All other determinations listed in table 1, with the exception of those on the Yolo clay, were made in two runs of the centrifuge, four centrifuge cups being filled at each run for each size of sample. The values for the samples dried in the centrifuge cups are seen to be greater than the corresponding values for samples of the same size made in another run of the centrifuge and removed from the cups, the differences being larger than can be accounted for by the water retained in the filter paper.

The 40, 50, and 60-gm. samples of the Yolo clay do not show a decrease in moisture content with increased amounts of soils used in the centrifuge. This is probably due to the effect reported by Joseph and Martin.<sup>9</sup> Water was found to be standing on the surface of these samples when the centrifuge was stopped. It was noted, however, that when these clay samples were placed in the moist chamber after centrifuging, the water which stood on the surface of the soil in the centrifuge cups was quickly reabsorbed. This suggests that the soil may have a certain resiliency. It should be noted, however, that the 10, 20, and 30-gm. samples of this soil showed a decrease in moisture retained, with an increase in size of sample.

In spite of the exceptions noted there is unquestionably a marked relation between the percentage of moisture retained and the amount of soil used in making the determination. An inspection of the probable error of the means of the values obtained clearly indicates that these differences are decidedly significant.

The Yolo clay loam was explored throughout a greater range of variation in weight of sample than were the other soils, the samples varying from 5 to 300 gm. The results of these tests are illustrated in figure 2. Samples up to and including 60 gm. were centrifuged in the standard centrifuge cups, the data for these determinations being given in the second line of table 1.

It was found impossible to use a larger sample than 60 gm. of air-dried soil of this type in the standard cups without undue packing of the soil. Special collapsible boxes, illustrated in figure 5, described later, were therefore used for the larger samples. Three hundred gm. of air-dried soil were the maximum which could be used in these boxes without packing. Only four of these boxes could be used at one time



in the centrifuge. The averages of four determinations for the 120-gm. samples of Yolo clay loam gave  $21.7 \pm 0.02$  per cent; for the 180-gm. samples,  $20.6 \pm 0.09$  per cent; for the 280-gm. samples,  $20.6 \pm 0.06$  per cent; and for the 300-gm. samples,  $19.1 \pm 0.06$  per cent. The 120, 180, and 280-gm. samples were centrifuged for one hour and the 300-gm. sample for two hours. These large samples were removed from the boxes after centrifuging and the filter paper discarded before making the moisture determinations.

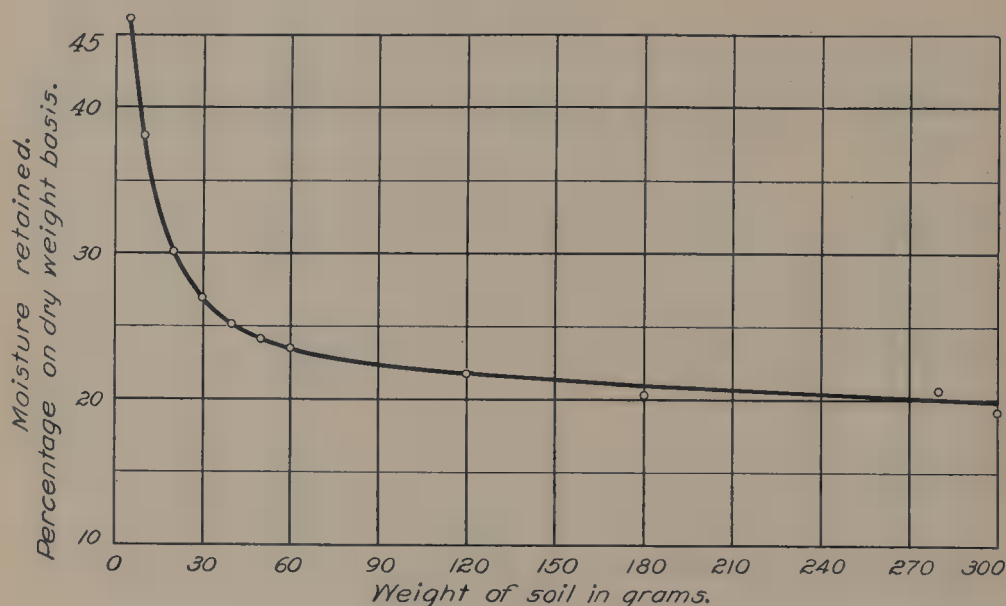


Fig. 2. Percentage of moisture retained by centrifuged samples of Yolo clay loam soil varying in weight from 5 to 300 gm.

#### DURATION OF CENTRIFUGE RUN

To test the effect of duration of run on the percentage of moisture retained by the Yolo clay loam, 30-gm. samples of air-dried soil were used. Averages of the sixteen determinations for each period of time used are presented in table 3 A. The samples were not removed from the centrifuge cups. The moisture determinations, therefore, include the moisture retained in the filter paper.

No apparent differences were obtained in the percentage of moisture retained in the 5, 10, and 15-minute tests, but the 30-minute run seemed to produce a decrease in moisture content. This, again, may be a variation due to the fact that these tests were made at different times.



TABLE 3\*

INFLUENCE OF TIME OF CENTRIFUGING ON THE PERCENTAGE OF MOISTURE RETAINED

- A. Samples retained in centrifuge cups in making moisture determinations, average of sixteen determinations.
- B. Filter paper discarded, average of eight determinations.
- C. Covers of centrifuge cups sealed with paraffine, filter paper discarded, average of eight determinations.

Date of test	Weight of air-dried samples in grams.	Weight of oven-dried samples in grams.	Duration of centrifuging in minutes	Percentage of moisture retained
--------------	---------------------------------------	--	-------------------------------------	---------------------------------

A. Samples retained in centrifuge cups.

1922				
September 23.....	30.0	28.8	5	27.8±0.03
September 26.....	30.0	28.7	10	27.8±0.04
September 26.....	30.0	28.7	15	27.6±0.05
May 11.....	30.0	29.0	30	26.9±0.05

B. Filter paper discarded.

1924				
May 19.....	30.0	29.1	5	26.6±0.03
May 19.....	30.0	29.0	10	26.6±0.02
May 19.....	30.0	29.1	15	26.4±0.03
May 21.....	30.0	28.9	30	26.3±0.03

C. Covers of centrifuge cups sealed with paraffine, filter paper discarded.

May 25.....	30.0	28.8	10	27.1±0.03
May 25.....	30.0	28.8	20	26.6±0.02
May 25.....	30.0	28.7	30	26.4±0.04
May 25.....	30.0	28.8	60	26.1±0.02

\* This table was made and discussed after the paper was originally submitted for publication.

The influence of the time of centrifuge run on the percentage of moisture retained was again tested at a later date, using 30-gm. samples of air-dried Yolo clay loam. However, in this test the samples were removed from the centrifuge cups and the filter paper discarded before making the moisture determinations. The average of eight determinations for each period of time is given in table 3 B. There appears to be a slight decrease in percentage of moisture retained with increase in time of centrifuging.

A further test was made of Yolo clay loam, again using 30-gm. air-dried samples. The periods of time of centrifuging ranged from

10 to 60 minutes. The samples were then removed from the cups and filter papers discarded before making moisture determinations. This test differed from the two former tests in that the covers of the cups were sealed with a coating of parafine before they were placed in the centrifuge. This prevented any drying of the soil in the cups, which might have been induced by the movement of air through the samples. Such a movement of air might possibly take place if the speed of the centrifuge varied after it had attained the desired number of revolutions per minute.

The results of this test, recorded in table 3 C, likewise indicate that there is a decrease in moisture content with increase in time of centrifuging. The fact that these samples retained a greater amount of moisture than those in the unsealed cups suggests that possibly, drying due to movement of air through the samples, may have some effect on the moisture equivalent.

Even if it is assumed that there is no decrease in per cent moisture retained with this increased time of centrifuging, it does not necessarily follow that equilibrium of the moisture within the blocks of soil has been established with the shorter runs.

#### INFLUENCE OF FILTER PAPER ON PERCENTAGE OF MOISTURE RETAINED

Sixteen of the filter papers used to cover the wire gauze bottom of the centrifuge cups were placed in the cups and, after being wet, were centrifuged for 30 minutes at the standard speed. The loss in weight after drying for 36 hours at 110° C. was  $0.31 \pm 0.002$  gm. That is, the filter papers retained 0.31 gm. of water against the centrifugal force. This test was repeated later with the same result.

Moisture determinations were made simultaneously on sixteen 30-gm. samples of air-dried Yolo clay loam, eight with filter papers and the other eight without them. The average percentage of moisture retained by the eight samples with filter paper was  $26.9 \pm 0.09$ . The average weight of oven-dried soil in these samples was 28.8 gm. The other eight samples averaged  $25.6 \pm 0.08$ . The oven-dried samples averaged 28.4 gm. These sixteen samples were not removed from the centrifuge cups in making the moisture determinations. They were moistened again, and after standing for 24 hours, were again centrifuged. The second tests gave an average percentage of moisture

retained of  $25.7 \pm 0.08$  for the samples with the filter paper, and  $24.7 \pm 0.10$  for the others. The percentage of moisture retained by the samples with filter paper was  $1.3 \pm 0.11$  greater than for those without filter paper.

An additional sixteen samples of Yolo clay loam were centrifuged at a later date. All the samples were provided with filter paper. Eight of the samples were removed from the cups, the filter paper discarded, and moisture determinations made in the usual manner. The average percentage of moisture retained was  $26.4 \pm 0.05$ . The average dried soil in a sample was 28.6 gm. The remaining eight samples were retained in the centrifuge cups and dried. The percentage of moisture retained was  $27.1 \pm 0.03$ . The average oven-dried soil in a sample was 28.9 gm. The difference in percentage of moisture retained for these two sets of samples was  $0.7 \pm 0.06$ .

The amount of moisture retained by the filter paper against the centrifugal force and the pressure exerted by the weight of the soil on the filter paper was determined in a few instances. Pieces of filter paper lost an average of  $0.30 \pm 0.01$  gm. on drying after being centrifuged and having on tops, an average weight of 4.84 gm., which was the average oven-dried weight of 5 gm. of air-dried soil. Filter paper, subjected to a superimposed weight of 17.1 gm. and centrifuged, lost  $0.26 \pm 0.005$  gm. An average superimposed weight of 29.8 gm. and centrifuging gave an average loss of weight in drying of the filter paper of  $0.26 \pm 0.01$  gm.

These data which indicate the effect on the moisture equivalent by the amount of water retained by the filter paper against the centrifugal force are given in the following tabulation:

Conditions under which test was made.	Grams of water retained	Percentage moisture retained.
Filter paper centrifuged alone.....	$0.31 \pm 0.002$	
Filter paper with 4.84 gm. soil on top.....	$0.30 \pm 0.01$	
Filter paper with 17.1 gm. soil on top.....	$0.26 \pm 0.005$	
Filter paper with 29.8 gm. soil on top.....	$0.26 \pm 0.001$	
Samples centrifuged at same time:		
Soil with filter paper.....		$26.9 \pm 0.09$
Soil without filter paper.....		$25.6 \pm 0.08$
Samples centrifuged at same time:		
Soil with filter paper retained.....		$27.1 \pm 0.03$
Soil with filter paper discarded.....		$26.4 \pm 0.05$

## DURATION OF WETTING

The effect of the time during which the soil is kept moistened before centrifuging, on the percentage moisture retained is illustrated by the data in table 4, and the curves in figure 3. Yolo clay loam soil was used in these trials. Sixteen determinations for each size of sample were made together, the samples not being removed from the

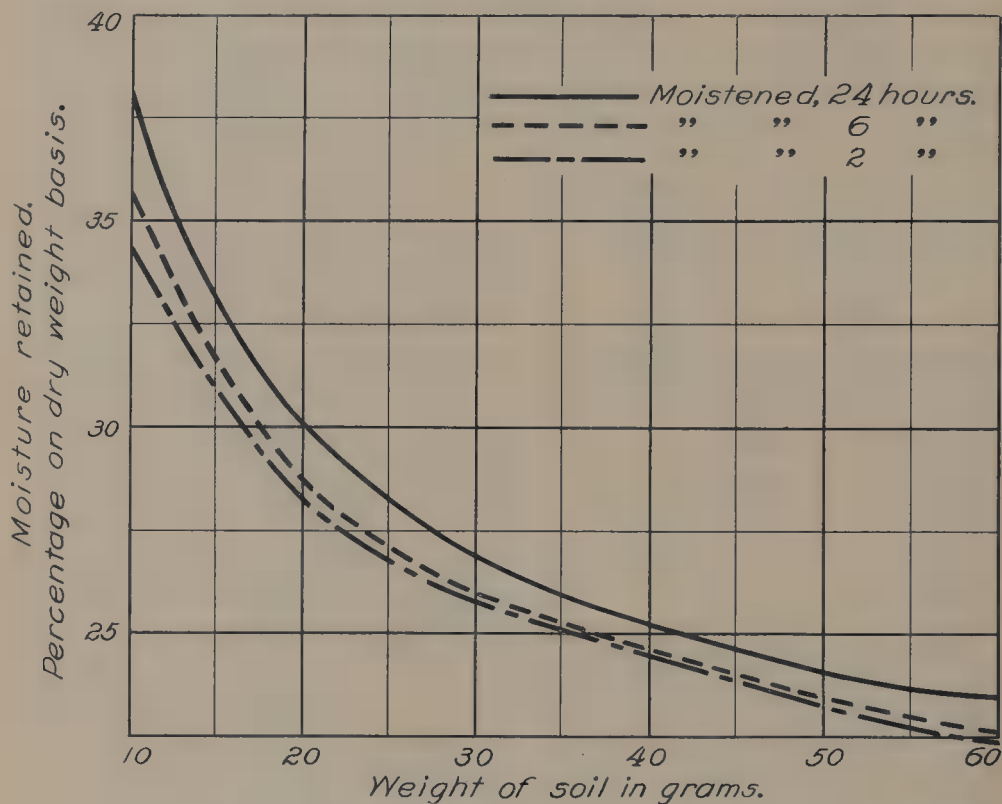


Fig. 3. The effect of the length of moistening period upon the percentage of moisture retained by various weights of samples of Yolo clay loam soil.

centrifuge cups in making the moisture determinations. The preliminary wettings were for periods of 2, 6, and 24 hours. The data of table 4 clearly indicate a significant difference in the percentage of moisture retained for the 24-hour wetting and the 2- and 6-hour wettings. It is also to be noted that in every instance the percentage moisture retained decreased when the amount of soil used in the determination was increased.

Since distilled water was used to moisten, though never in such excess that leaching would take place, these differences cannot be attributed to the effect reported by Sharp and Waynick.<sup>11</sup>

TABLE 4

INFLUENCE OF THE TIME DURING WHICH YOLO CLAY LOAM WAS KEPT MOISTENED BEFORE CENTRIFUGING ON THE PERCENTAGE OF MOISTURE RETAINED\* AS DETERMINED BY THE USE OF SAMPLES OF DIFFERENT WEIGHT

Number of hours soil was kept moistened.	Number of determinations for each size sample.	Weights of samples of air-dried soil in grams.					
		10	20	30	40	50	60
2	16	34.3 ±0.15	28.2 ±0.05	25.8 ±0.06	24.4 ±0.02	23.3 ±0.02	22.5 ±0.01
6	16	35.6 ±0.06	28.7 ±0.06	26.0 ±0.03	24.6 ±0.05	23.4 ±0.03	22.6 ±0.06
24	16	38.1 ±0.12	30.1 ±0.04	26.9 ±0.05	25.1 ±0.08	24.1 ±0.02	23.5 ±0.02

\* These samples were dried in centrifuge cups and therefore include the moisture in the filter paper.

INFLUENCE OF VARIATION OF CROSS-SECTIONAL AREA

The effect on the percentage of moisture retained by varying the weight of soil per unit of cross-section is further indicated in the following test. Paraffined wooden blocks were placed in the sixteen cups which made up one full set for centrifuging. The wooden blocks fitted precisely one-half of the cups. Filter paper was used on the half of the cup not covered by the wooden blocks. In each of eight cups, 15 gm. of air-dried Yolo clay loam was placed, and each of the remaining eight cups of the set was filled with 30 gm. of the same soil. Since the paraffined wooden blocks occupied just one-half of the bottoms of the cups, the 15-gm. samples had the same thickness as normal 30-gm. samples and the 30-gm. samples here used had the same thickness as normal 60-gm. samples. These sixteen samples were centrifuged, the wooden blocks removed, and the moisture content of the samples determined without removing the soil from the cups. The average weight of oven-dried soil in the 15-gm. air-dried samples was 14.38 gm. The average percentage of moisture retained by the eight samples was found to be  $27.81 \pm 0.05$ . The average weight of oven-dried soil in the 30-gm. samples was 28.76 gm. The average percentage of moisture retained by these eight samples was  $23.65 \pm 0.02$ .

It will be seen that the percentage of moisture retained for the 30-gm. samples, each of which occupied one-half of the cup, agreed



very closely with the value obtained for the 60-gm. samples of this same soil given in table 1. The average value for the 15-gm. samples in one-half of the cup is somewhat higher than the value for the 30-gm. samples given in table 1, but agrees closely with the values for the 30-gm. samples run for 5, 10 and 15 minutes given in table 3 A.

These results are further evidence that the weight of soil per unit of cross-section has an intimate relation to the percentage of moisture retained. It further suggests that there is no variation in the percentage of moisture retained normal to the radii of the centrifuge.

## SECTION II

### DISTRIBUTION OF MOISTURE WITHIN THE SAMPLES

The rapid decrease in percentage of moisture retained with increase in weight of sample, as reported above, led us to ascertain the amounts of moisture held by thin layers of the sample having their centers at different distances from the axis of rotation. This was accomplished by slicing the samples approximately normally to the radius and drying the several slices or layers separately. The distribution of water within the sample thus observed was clearly influenced by the movement of moisture in the soil during the time elapsing between the moment the electric switch was disconnected to stop the centrifuge until the slicing was completed. Measurements of the rate of readjustment of the moisture during a three-hour period after the centrifuge was stopped and reported in table 19, in connection with which this question is more fully considered, lead us to believe that the amount of readjustment during the period of stopping the centrifuge is relatively small. Samples of 31-, 60-, 280-, and 300-gm. air-dried soil were thus studied by slicing into layers varying in thickness from 2 mm. to 10 mm. At the outset we endeavored to divide 31-gm. air-dried samples into two layers of equal depth with a spatula, after taking them out of the centrifuge cup. This method of approximate division was later replaced by two specially designed microtomes, one for samples up to 62 gm., and one for the 280 and the 300-gm. samples.



MOISTURE CONTENT OF INNER AND OUTER HALVES OF  
THE SAMPLES

Two types of soil, namely Yolo clay loam and Oakley fine sand, were used for the 31-gm. samples, which were divided into inner and outer halves with a spatula. Eight determinations were made on the Yolo clay loam, of which the average moisture equivalent for the entire sample was 26.2 per cent. The inner half of the sample contained 25.5 per cent, and the outer half 26.9 per cent of moisture. Eight determinations were also made on the Oakley fine sand, the average moisture equivalent for the entire sample being 6.0 per cent; for the inner half, 5.3 per cent; and for the outer half, 6.8 per cent. In every determination for each soil, the moisture content of the inner half of the sample was less than that of the outer half.

## DISTRIBUTION OF MOISTURE IN SUCCESSIVE LAYERS

In order to be able to determine more exactly the distribution of moisture within a sample, a special microtome was used, which made it possible to slice the soil into 2-mm. layers with precision. (See plate 1, figure 1.)

*Description of the Microtome.*—This microtome was designed and constructed by Mr. E. J. Hoff, of the Division of Agricultural Engineering, Bureau of Public Roads, U. S. Department of Agriculture. It consists of a cup 2 cm. deep, having the same inside cross-section dimensions as the centrifuge cup. The bottom of the cup is a piston with one curved surface having a radius of 15.24 cm., to fit the curvature of the soil sample formed by the centrifuge cup. This piston is mounted on a micrometer screw having a thread pitch of 0.8 revolutions per millimeter. The two parallel top edges of the sides of the cup are concave and have the same radius as the piston bottom, thus making it possible to slice the soil at a constant distance from the axis of rotation of the centrifuge. A small sharp-edged slicing pan was used, as illustrated in plate 1, figure 1.

*Method of Using the Microtome.*—Immediately after stopping the centrifuge, the cups were placed in a moist chamber. They were then taken out one at a time, the soil block carefully removed and placed in the microtome, the depth of which was set precisely at 16 mm. at the start. The inner surface of the soil block protruded from 1 to

2 mm. above the sides of the microtome, as the blocks were from 17 to 18 mm. thick. Since it was impracticable to make the inner surface exactly smooth, or to make the sample of uniform depth, the first layer ranging from 1 to 2 mm. in depth was discarded. The microtome screw was then turned 1.6 revolutions, thus advancing the soil block 2 mm. The first slice for moisture determination was then obtained, quickly put into a weighing bottle or can, covered, and placed in a saturated atmosphere until weighed on an analytical balance. It was found necessary to press the soil block lightly while slicing in order to hold it firmly against the curved piston and to assure uniformity in thickness of successive layers. This was especially necessary when the soil block became very thin. In some cases it was quite difficult to slice the last 4 mm. into layers of equal thickness.

*Moisture Content in Layers of Different Thicknesses of Two Soils.*—Two soils were used in the first trial with the special microtome, and satisfactory layers were obtained from the blocks taken from eleven cups. The results of the moisture determinations for these layers are presented in table 5.

It will be noted that in both the Yolo clay loam and the Yolo silt loam there is a marked tendency for the moisture content to increase in passing through the soil blocks from the inner to the outer surface. Notwithstanding some exceptions to this general tendency, it is significant that, in every case, the moisture content of the outer layer is appreciably greater than that of the inner. In the Yolo clay loam, the differences between the outer and inner layers are 2.7 per cent moisture for the 2-mm. samples, 2.3 per cent for the 3-mm. samples, and 2.0 per cent for the 4-mm. samples. In the Yolo silt loam, the differences for samples of the same thickness are 1.9, 1.3, and 1.3 per cent, respectively. The data presented in table 5 warrant the conclusion that the moisture content increases as the distance of the successive layers from the axis of rotation increases.

*Moisture Content in Layers of Equal Thickness of One Soil.*—In order to decrease the magnitude of unavoidable errors of observations, fourteen 62-gm. air-dried samples of one soil, Yolo clay loam, were used and, after centrifuging, each was sliced into 2-mm. layers. The first slice, being of irregular surface, was rejected as in the preliminary tests.

TABLE 5

PRELIMINARY DETERMINATIONS OF MOISTURE DISTRIBUTION WITHIN 60-GM. SAMPLES AFTER BEING CENTRIFUGED

Results expressed in percentage of moisture on basis of oven-dry soil.  
(Layer No. 1 is nearest the axis of rotation, No. 8 farthest from it.)

Thick-ness of slice	Yolo clay loam.								
	Number of layer.....	1	2	3	4	5	6	7	8
2-mm.	{ First trial.....	20.2	20.8	21.1	21.2	21.4	21.3	21.8	22.6
	{ Second trial.....	20.3	20.8	21.6	21.2	22.1	21.8	22.2	23.2
	Averages.....	20.2	20.8	21.4	21.2	21.8	21.6	22.0	22.9
3-mm.	{ First trial.....	20.3	21.4	22.4	22.2	23.4			
	{ Second trial.....	20.3	21.5	21.8	21.7	22.9			
	Averages.....	20.3	21.4	22.1	22.0	22.6			
4-mm.	{ First trial.....	21.4	22.4	22.9	23.3				
	{ Second trial.....	21.1	21.6	22.8	23.0				
	Averages.....	21.2	22.0	22.8	23.2				
Yolo silt loam.									
	Number of layer.....	1	2	3	4	5	6	7	8
2-mm.	Only trial.....	21.7	22.6	22.9	22.8	23.1	23.2	22.9	23.6
3-mm.	{ First trial.....	22.1	23.1	23.2	24.1	23.9			
	{ Second trial.....	22.9	23.2	23.4	23.1	23.8			
	Averages.....	22.5	23.2	23.3	23.6	23.8			
4-mm.	{ First trial.....	23.0	23.5	24.2	23.9				
	{ Second trial.....	22.4	23.7		24.1				
	Averages.....	22.7	23.6	24.2	24.0				

The moisture distribution in each of the fourteen samples which were successfully divided into eight 2-mm. layers is reported in table 6. Faulty manipulation damaged the samples in cups 9 and 16 so that they could not be used for slicing to give accurate results.

In the averages of the fourteen determinations for each layer it will be noted that the moisture content increased continuously from

the first to the fifth; that in the fifth, sixth, and seventh layers it was substantially constant, and that it increased from the seventh to the eighth layer.

TABLE 6

DETERMINATIONS OF MOISTURE DISTRIBUTION WITHIN FOURTEEN 60-GM. SAMPLES OF YOLO CLAY LOAM LAYERS SLICED WITH PAN AFTER BEING CENTRIFUGED

Results expressed in percentage of moisture on basis of oven-dry soil.

(Layer No. 1 is nearest the axis of rotation, No. 8 farthest from it.)

Thick- ness of layer.	Cup num- ber	Number of layer—							
		1	2	3	4	5	6	7	8
2 mm.	1	19.97	20.53	21.01	21.55	21.64	21.64	21.77	23.09
2 mm.	2	20.33	20.31	20.86	21.14	21.38	21.40	21.53	22.90
2 mm.	3	20.41	20.41	20.98	21.24	21.24	20.98	19.85	22.69
2 mm.	4	20.29	20.43	20.90	21.06	21.39	21.48	21.42	22.84
2 mm.	5	20.19	20.30	20.82	20.94	21.03	21.06	21.33	22.94
2 mm.	6	20.10	20.16	20.66	20.89	21.07	20.84	20.98	22.10
2 mm.	7	20.39	20.63	20.33	21.04	21.09	20.81	20.81	22.14
2 mm.	8	18.70	20.33	20.80	20.95	21.17	21.33	21.19	22.77
2 mm.	10	19.27	20.28	20.83	21.02	21.22	21.44	21.25	22.77
2 mm.	11	20.50	20.42	20.99	21.18	21.38	21.47	21.38	22.62
2 mm.	12	20.38	20.67	20.98	21.03	21.19	21.29	21.34	22.61
2 mm.	13	20.36	20.57	20.84	20.86	21.34	21.33	21.37	22.79
2 mm.	14	20.55	20.79	19.45	21.42	21.64	21.67	21.30	22.72
2 mm.	15	20.56	20.78	21.02	21.58	21.63	21.41	21.59	23.14
Averages.....		20.14	20.48	20.75	21.14	21.33	21.30	21.22	22.72
		±0.10	±0.03	±0.07	±0.04	±0.04	±0.05	±0.08	±0.05

#### MODIFIED MICROTOME METHOD OF SLICING SAMPLES

Further determinations of moisture distribution within 60-gm. samples made by a slightly modified method of slicing are reported in table 7.

An attempt was made, in slicing the layers for which moisture distribution is reported in table 6, to recover for each layer every particle of soil in order that the apparent specific gravity might be calculated from the computed volume and the weighed amount of soil in each layer. This was found impracticable by the method described above because of the breaking of the soil blocks at the corners, and because of the loss from the sides which occurred in removing the samples from centrifuge cups. However, this was accomplished by

TABLE 7

DETERMINATIONS OF MOISTURE DISTRIBUTION WITHIN EIGHT 60-GM. SAMPLES OF  
YOLO CLAY LOAM AFTER BEING CENTRIFUGED, AND COMPARISONS WITH  
AVERAGES OF TESTS REPORTED IN TABLE 6

Results expressed in percentage of moisture on basis of oven-dry soil.  
(Layer No. 1 is nearest the axis of rotation, No. 8 farthest from it.)  
(These determinations were made by more precise methods than those of  
table 6, using a razor and special guides on the microtome.)

Layers	Cup number	Number of layer						
		1	2	3	4	5	6	7 and 8
2 mm.....	34	19.64	20.61	20.67	21.11	21.37	21.75	22.03
2 mm.....	17	19.68	20.86	20.71	21.23	21.71	21.73	22.32
2 mm.....	2	19.74	20.20	20.79	21.20	21.56	21.57	21.84
2 mm.....	9	18.78	20.38	20.86	20.01	21.23	21.36	21.93
2 mm.....	19	19.91	20.23	20.86	21.42	21.84	21.98	21.90
2 mm.....	13	19.70	20.12	20.61	21.07	21.49	21.75	22.30
2 mm.....	8	19.91	20.45	20.90	21.26	21.81	22.04	21.91
2 mm.....	12	19.90	19.95	20.86	21.19	21.24	21.78	22.13
Averages.....		19.66	20.35	20.76	21.19	21.59	21.75	22.04
		±0.09	±0.07	±0.03	±0.03	±0.05	±0.05	±0.04

For graphical illustration see figure 4.

Averages of two sets of tests:

Above averages.....	19.66	20.35	20.76	21.19	21.59	21.75	22.04
Table 6.....	20.14	20.48	20.75	21.14	21.33	21.30	21.97
Means of averages.....	19.90	20.42	20.76	21.16	21.46	21.52	22.00
	±0.07	±0.04	±0.04	±0.03	±0.03	±0.04	±0.05

using a one-piece cardboard lining with waxed surfaces. The lower edges of the linings were curved so as to fit the bottoms of the cups snugly and were placed around the sides of the cups. The filter papers were then placed in the bottoms and the soil samples weighed into them. The cardboard linings were wide enough to extend about 6 mm. above the centrifuge cups. This made it possible to remove the linings, together with the block of soil, without loss or breaking at the corners. Furthermore, the layers for which the percentage of moisture is reported in table 7 were sliced by using a razor instead of the special cutting pan prepared for use with the microtome. The razor was of necessity advanced downward from the top of the curved guides of



the special cup. Parallel guides cut to the same radius as the concave top edges of the microtome were clamped to the sides of the microtome cup. These made it possible to press the back of the razor against the guides and thus to avoid variations in thickness of slice due to differences in the manner of holding the razor. This arrangement of guides and their use are illustrated in plate 2, figure 2, and plate 1, figure 2. Because this motion is opposite in direction to that of the cutting pan, greater difficulty was found in separating the last two lawers. Layers

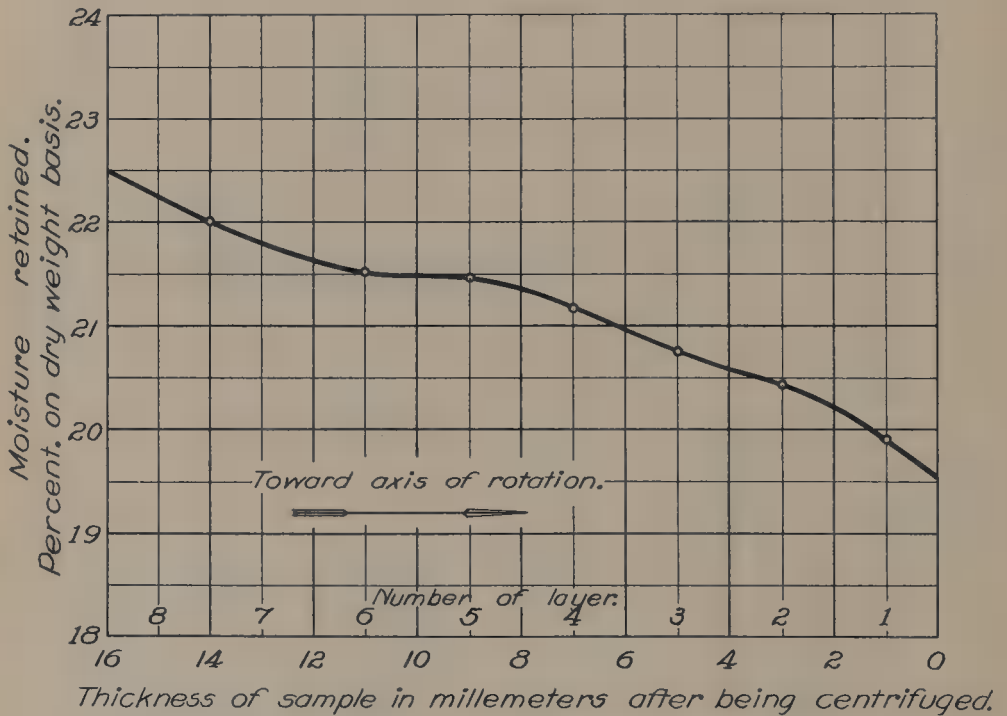


Fig. 4. Moisture distribution within 60-gm. samples of Yolo clay loam soil after being centrifuged.

7 and 8 therefore are reported as one layer 4-mm. thick. All of the moisture percentages in table 7 were made in weighing bottles.

Upon examination of the average moisture percentages in the several layers as given in table 7, it will be noticed that the moisture content increased continuously in every case from layer 1 to 8. Moreover, the means of the averages of the two sets of determinations, given at the bottom of table 7, likewise show a continuous increase, the magnitude of the increase from the inner to the outer layer being 2.10 per cent moisture, or 10.5 per cent of the moisture content of the inner layer. A clear picture of the rate of moisture increase as the

distance of the layer from the axis of rotation increases may be obtained by examining figure 4. The curve connecting the several points seems to approach very closely to a straight line. The theoretical interpretation of this curve is considered in the discussion of results presented in Section V of this paper.

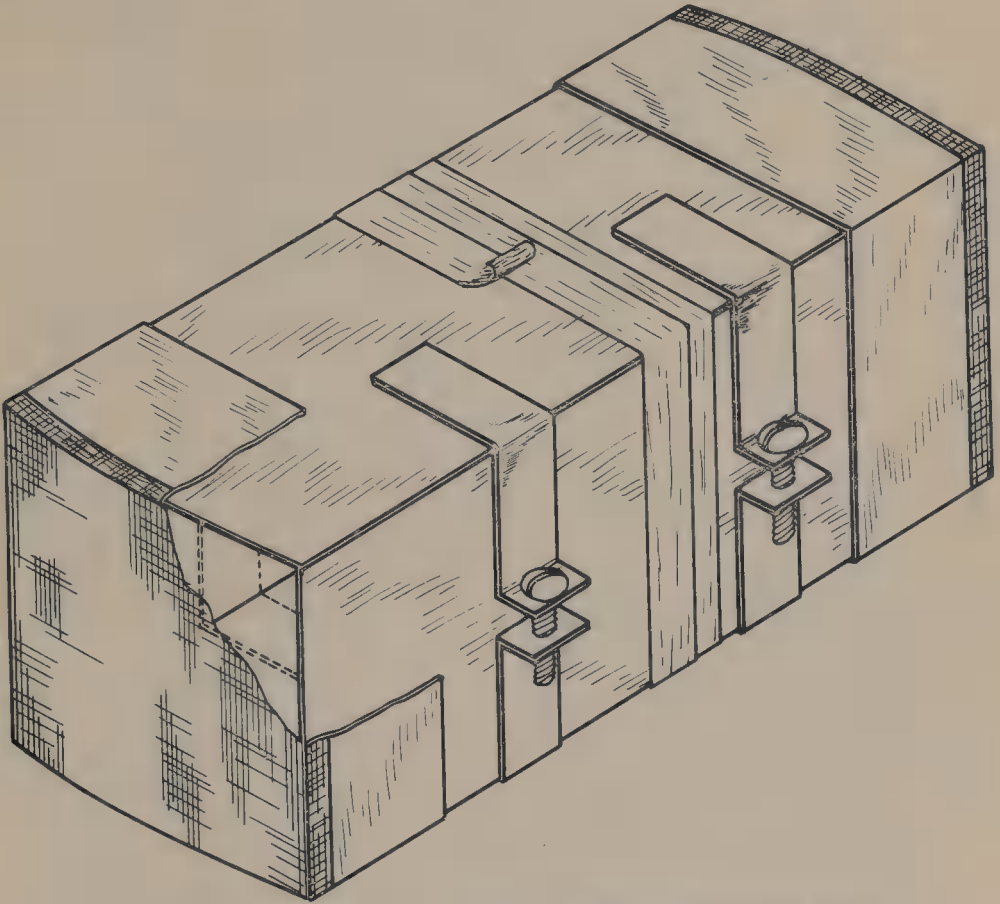


Fig. 5. Collapsible cup used for centrifuging weights of soil greater than 60 gm.

#### DISTRIBUTION OF MOISTURE WITHIN LARGE SAMPLES

To add further to our information concerning this law of distribution of moisture within the centrifuged blocks of soil, the distribution of moisture in two large samples was measured, one containing 280 gm., the other 300 gm. To do this it was necessary to devise special collapsible centrifuge cups. These cups, illustrated in figure 5, were made of two pieces of 22-gage sheet metal, approximately rectangular, having dimensions of 11.5 by 11.5 cm., each piece turning through a right angle about its longitudinal axis through its center

point. These two pieces were fitted together so as to make a rectangular box just large enough to fit inside the standard centrifuge cup. The edges of the pieces forming the sides of the cup were drawn tightly together by means of straps and screws as shown in figure 5. The collapsible cup was carefully wrapped with tire tape, lined with thin

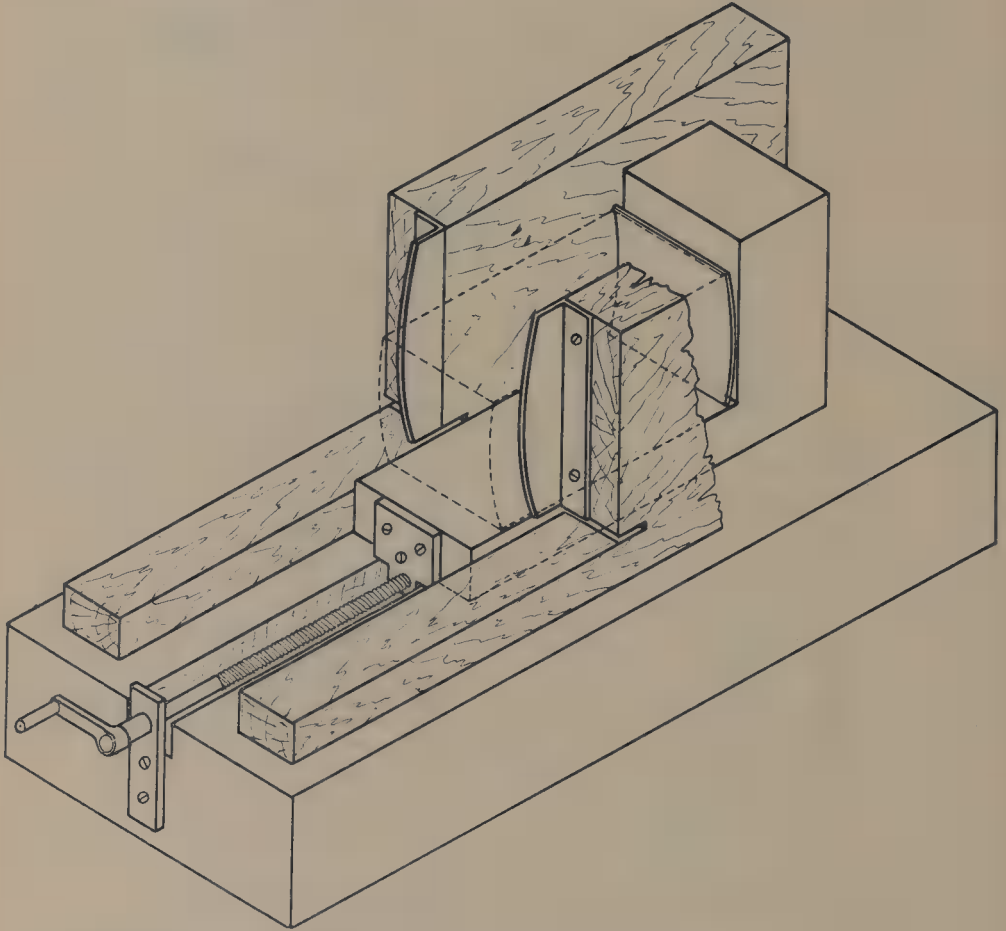


Fig. 6. Special macrotome for slicing 280 and 300-gm. samples of centrifuged soil.

paper, and fitted into the standard cup which formed the outer end of the special cup. The weighed sample of soil was placed carefully in the cup and moistened with distilled water for twenty-four hours. Standard cups were fitted over the inner ends of the special cups before the latter were placed in the machine, to prevent their being spread apart by the soil pressure. Four of these special cups were used in each determination of the large samples. After removal from the centrifuge, the tire tape was cut away from the sides of the special

cup and the standard cups removed from either end. This made it easy to remove the walls of the special cup from the block of soil without cracking it or breaking off the corners.

*Description and Use of the Macrotome.*—The large block was placed in a special macrotome (fig. 6). This consists, essentially of a sliding carriage, traveling between two upright guides. The dimensions of this carriage and the consequent spacing of the guides are such that the block of soil fits snugly between them. There is, however, just enough play to allow the block of soil to be moved forward without scraping. A concave brass plate with a radius of 15.24 cm. is attached to the block, forming the end of the carriage. This plate has the same radius as the convex brass cutting guides attached to the front ends of the upright guides. The movement of the carriage is controlled by means of a micrometer screw having the same pitch as that of the micrometer previously described. The layers can be cut precisely to any thickness desired. A razor was used to slice the soil, its downward motion being guided by the convex plates. A tray, not shown in figure 6, was used to catch the soil as it was cut from the block. This was made of celluloid and was inserted under the block of soil and extended a short distance back from the convex guide plates. The recesses cut into the upright guides and into which the bottom of the celluloid tray fitted are shown in the text figure.

The 280-gm. sample was kept in the machine at the full speed of 2440 r.p.m. for one hour. The moisture distribution after this period of operation is reported in table 8 A and in figure 7. Layers 1 to 13 were each precisely 5 mm. thick, but layer 14, the last one taken off the macrotome, varied from 5 to 7 mm.

It will be noted in examining figure 7, that the moisture content in the 280-gm. sample tended to increase from the inner layer, number 1, to layer number 6; that it was substantially the same in layers 6 and 7; that it tended to decrease slightly from layers 7 to 12 and to increase from layers 12 to 14. While these data do not show significant differences in every case, yet the tendencies appear to be as indicated above.

The moisture distribution in the 300-gm. samples after two hours in the centrifuge is given in table 8 B, and figure 7. Minor modifications of the macrotome made it necessary to cut off layer 1 first, and also to leave layer 2, the last one cut, thicker than the others. Layers 3 to 15 were each 5 mm. thick, and layers 1 and 2, 8 to 15 mm. thick.

TABLE 8

DISTRIBUTION OF MOISTURE (A) WITHIN A 280-GM. SAMPLE OF YOLO CLAY LOAM AFTER BEING CENTRIFUGED ONE HOUR, AND (B) WITHIN A 300-GM. SAMPLE OF THE SAME SOIL AFTER BEING CENTRIFUGED TWO HOURS

(Layer No. 1 is nearest the axis of rotation.)

A.—Size of sample, 280 gm. Time in centrifuge, one hour.							
Thickness of layer, mm.	Number of layer	Number of cup used*				Average	
		1	2	3	4		
7	1	19.20	19.64	19.21	.....	19.35	±0.10
5	2	20.42	19.45	19.90	19.52	19.82	±0.13
5	3	20.11	19.44	19.81	20.42	19.94	±0.16
5	4	21.12	19.84	20.39	20.16	20.38	±0.15
5	5	20.79	20.35	20.76	20.78	20.67	±0.07
5	6	20.72	21.31	21.43	21.75	21.30	±0.15
5	7	21.05	21.61	21.09	21.40	21.29	±0.09
5	8	21.05	21.56	20.16	21.22	21.00	±0.07
5	9	20.80	20.88	21.10	21.48	21.07	±0.10
5	10	21.12	20.85	20.41	20.73	20.78	±0.10
5	11	21.22	20.39	20.49	20.52	20.65	±0.13
5	12	20.25	20.03	20.80	20.53	20.40	±0.11
5	13	21.18	20.45	20.54	20.33	20.62	±0.12
4 to 6	14 and 15	21.13	20.41	20.29	20.77	20.65	±0.13

\* For graphical illustration see figure 7.

B.—Size of sample, 300 gm. Time in centrifuge, two hours.							
Thickness of layer, mm.	Number of layer	Number of cup used*				Average	
		1	2	3	4		
8 to 15	1 and 2	16.40	16.76	17.31	17.46	16.98	±0.17
5	3	18.58	17.74	17.41	18.30	18.01	±0.18
5	4	17.83	17.94	17.65	19.09	18.14	±0.23
5	5	19.09	17.76	18.17	18.37	18.35	±0.19
5	6	18.54	18.56	18.35	19.06	18.63	±0.10
5	7	18.97	19.13	19.58	19.10	19.20	±0.09
5	8	20.26	19.38	18.96	20.14	19.68	±0.20
5	9	20.92	19.19	19.45	19.00	19.64	±0.29
5	10	20.42	19.27	19.19	19.47	19.59	±0.19
5	11	20.40	19.72	20.92	20.21	20.31	±0.17
5	12	19.67	19.31	19.86	20.63	19.87	±0.19
5	13	19.49	19.44	20.02	20.20	19.79	±0.13
5	14	19.25	19.42	19.70	19.21	19.40	±0.07
5	15	19.24	20.19	20.43	19.87	19.93	±0.17

\* For graphical illustration see figure 7.



Examination of figure 7 suggests that, ignoring slight irregularities, the moisture content in the 300-gm. sample increased from layers 1 to 11; that it decreased from layers 11 to 14; and that it increased from layers 14 to 15.

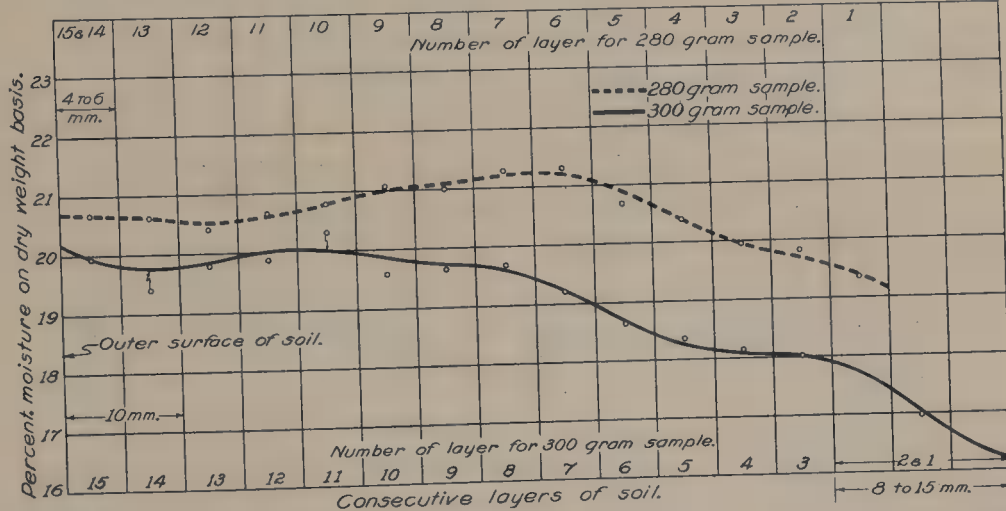


Fig. 7. Distribution of moisture within 280 and 300-gm. samples of Yolo clay loam soil. The 280-gm. samples were centrifuged one hour; the 300, two hours.

The difference in the forms of the two curves of figure 7 and their greater irregularity as compared to figure 4 probably reflects differences in compactness of soil and in the time during which the samples were in the centrifuge. It is probable that equilibrium had not yet been established in the large samples. Further discussion of the causes of these differences is given after the presentation of observations concerning the apparent specific gravity of soils after centrifuging and the influence of different pressures on the per cent of moisture retained.

### SECTION III

#### APPARENT SPECIFIC GRAVITY OF SAMPLES AFTER CENTRIFUGING

Determinations were made of the apparent specific gravity of blocks of soil after centrifuging. The volume of soil samples of different weights were separately determined. Two methods were used in making these volume determinations: First, the paraffine-immersion method wherein the volume of the centrifuged blocks of soil are immersed in a paraffine bath and the volume determined by the loss of weight in water, and second, direct-micrometer measure-

ment method in which the volume of the block of soil is determined by measuring with a micrometer the average thickness of the block, together with the cross-sectional area.

Similar results were obtained from the data secured in the experiments to ascertain the distribution of moisture in the blocks of soil after centrifuging as reported in Section II.

#### PARAFFINE-IMMERSION METHOD

Apparent specific gravity determinations of centrifuged samples of Yolo clay loam were made by the paraffine-immersion method. The essential details of the method as carried out are as follows: Four samples each of 10, 20, 30, and 60 gm. of air-dried Yolo clay loam were weighed and placed in the centrifuge cups in the usual manner. The samples were carefully smoothed and levelled in the manner previously described. They were moistened and allowed to stand for twenty-four hours. In order to avoid loss of soil in the centrifuge, pieces of filter paper were cut to fit the bottoms of the centrifuge cups accurately. As a further precaution, pieces of ashless filter paper were inserted between the cups and the wall of the centrifuge to catch any soil that might be thrown out, but this precaution proved unnecessary.

Before the samples were placed in the cups, the volumes of the cups without covers were determined by obtaining the loss of weight in water. The 10, 20, 30, and 60-gm. samples were carefully weighed. Samples of the same soil were taken to determine the amount of hygroscopic moisture present at the time the air-dried samples were weighed, and the amount of water-free or oven-dried soil calculated.

After centrifuging, the cups were placed in a moist chamber until weighed. The weight of the cup, plus the wet soil and filter paper in air was obtained. The cups with the soil samples were then placed in a paraffine bath until they were thoroughly coated with paraffine. Then the weight in air of the cup, plus the soil, filter paper, and paraffine was obtained. The difference between these two weights gave the weight of the paraffine added. The volume of the paraffine was calculated from the known density of the paraffine. The weight of the cup in water, plus the wet soil, filter paper, and paraffine coating was then obtained. The difference between the weight in air and the weight in water gave the volume of the cup plus the wet soil, filter paper, and paraffine. From the values for the volume of the cup, paraffine, and filter paper, the volume of the soil in the cen-

trifuge cup was calculated. The weight of the oven-dried soil in each sample divided by the volume gave the apparent specific gravity of the soil.

The volume of the filter paper in the bottom of the cups could not be directly determined. There may be a slight error introduced in the apparent specific gravity determinations of the different sized samples because of this fact. It is believed, however, that the difference between filter paper volume and that used, which was determined indirectly as described below, is very low. The volume of the filter paper was calculated as follows: fifty sheets of filter paper were wetted and pressed between two sheets of galvanized iron under a pressure of about 100 pounds. The thickness of the fifty sheets together was then measured and the volume of each sheet found to be 0.3 cc.

The averages of four apparent specific gravity determinations for each weight of sample, 10, 20, 30 and 60-gm., of air-dried Yolo clay loam are given in table 9.

TABLE 9

THE AVERAGE APPARENT SPECIFIC GRAVITIES OF THE 10, 20, 30 AND 60-GM. SAMPLES OF YOLO CLAY LOAM AS DETERMINED BY THE PARRAFINE-IMMERSION METHOD

(Each of the following values is the average of four determinations for each size sample.)

Weight of air-dried samples gm.	Weight of oven-dried samples gm.	Volume of soil in sample cc.	Apparent specific gravity
10.000	9.726	8.439	$1.16 \pm 0.025$
20.000	19.452	15.489	$1.26 \pm 0.011$
30.000	29.178	21.539	$1.35 \pm 0.013$
60.000	58.356	40.441	$1.44 \pm 0.003$

#### DIRECT-MICROMETER MEASUREMENT METHOD

Apparent specific gravity determinations of different sized samples of Yolo clay loam were made also by direct-micrometer measurements. The essential details of this method are as follows: Samples of the desired quantities of the soil were weighed and placed in the centrifuge cups, the bottoms of which were covered with pieces of filter paper. The surface of the soil in the cups was smoothed and levelled. The samples were then centrifuged in the usual manner and the cups

removed and placed in a moist chamber until used. Upon removal from the moist chamber, a thin piece of metal was placed on the surface of the soil in the centrifuge cup. This sheet of metal is the kind used for making shims. It is 0.003 inches or 0.008 mm. thick. It was cut to fit the inside of the cups with just enough clearance to allow it to be easily inserted and removed. It was used for the purpose of making micrometer measurements of the thickness of the layer of soil in the cup without allowing the micrometer screw to be forced into the moist soil. The metal was thin and flexible enough to allow it to adapt itself to the irregularities in the surface of the soil. It was divided into sixteen squares of equal dimensions, these being numbered consecutively from 1 to 16. A micrometer reading to 0.001 inches was used. The micrometer has a ratchet spring release so that a constant pressure was exerted each time a measurement was made.

The thickness of the soil, plus the thickness of the bottom of the centrifuge cup and the filter paper was obtained by taking readings at the center of each of the sixteen squares marked on the metal sheet. The soil was then carefully removed and placed in a weighing can and oven-dried to obtain the weight of water-free soil. The metal sheet was then placed on top of the filter paper in the centrifuge cup in the same position it had when it was placed on top of the soil. The thickness of the bottom of the cup, plus the thickness of filter paper, was measured at the centers of the sixteen squares. The difference in the averages of these two readings is the average thickness of the blocks of soil used as a sample. This method is the same in principle as the well known borrow-pit method used in surveying to determine the volume of earthwork. The thickness or depth of layer of soil obtained in this manner is the thickness measured radially, since the bottom of the centrifuge cup and the surface of the soil in the cup are concentric circles.

#### COSINE-METHOD OF ADJUSTING RESULTS

To compute accurately the volume by the principle of the previously described method, measurements should be made in planes parallel to the sides of the centrifuge cup. Since measurements of thickness were made radially normal to the circumference, each measurement divided by the cosine of the angle between the plane of measurement and the sides of the centrifuge cup gives the true thick-



ness. The two different angles between the radial plane of measurement and the sides of the centrifuge cup are  $7^{\circ} 00'$  and  $2^{\circ} 20'$ . If  $b$  represents the average of the measurements made in the radial plane of measurement making the angle of  $7^{\circ}$  with the sides of the cup, and  $c$  represents the average of those at the angle of  $2^{\circ} 20'$  the true average thickness of the sample of soil would be represented by

$$\frac{\frac{2b}{\cos 7^{\circ}} + \frac{2c}{\cos 2^{\circ} 20'}}{4}$$

But, since  $b$  and  $c$  are nearly equal, and the cosines of  $7^{\circ}$  and  $2^{\circ} 20'$  are nearly equal, only a negligible error is involved in letting  $a$ , the arithmetic mean of  $b$  and  $c$ , take the place of each. But  $a$  is the average thickness as measured by the micrometer. Hence the true thickness is approximately

$$\frac{\frac{a}{\cos 7^{\circ}} + \frac{a}{\cos 2^{\circ} 20'}}{2} \quad \text{or} \quad a \left[ \frac{\cos 7^{\circ} + \cos 2^{\circ} 20'}{2 \cos 7^{\circ} \times \cos 2^{\circ} 20'} \right]$$

or

$$a \times 1.0042.$$

The average measured thickness has in each case been corrected by multiplying it by the common factor 1.0042. The volume of each block has been obtained by multiplying the corrected average thickness by the area of the cross-section of the block of soil (24.216 sq. cm.) which was obtained by squaring the equal inside dimensions of the centrifuge cup.

The apparent specific gravity determinations by the direct-micrometer measurement method of various sized blocks of centrifuged Yolo clay loam and Oakley fine sand are given in table 10.

The specific gravity data presented in table 10 indicate a significant increase in apparent specific gravity as the weight of sample is increased. The differences for the Yolo clay loam are less marked than those found by the use of the paraffine-immersion method reported in table 9. A comparison of the probable errors of the determinations made by means of these two methods indicates that the direct-micrometer measurement method is the more accurate. Smaller differences were observed with the Oakley fine sand than with the Yolo clay loam. It seems safe to conclude, however, that the increase in weight above a 20-gm. sample causes some increase in compactness.



Apparent specific gravity determinations were also made by the micrometer method of 30-gm. air-dried samples of Yolo clay, Yolo silt loam, and Twin Falls silt loam. The average value of the four determinations for each of these soils, together with those of the 30-gm. samples of Yolo clay loam and Oakley fine sand are presented in table 11.

The data presented in table 11 seem to indicate that, for a sample of given weight, the apparent specific gravity increases when the size of the soil particles increases.

TABLE 10

APPARENT SPECIFIC GRAVITIES OF YOLO CLAY LOAM AND OAKLEY FINE SAND AFTER CENTRIFUGING WHEN DIFFERENT AMOUNTS OF SOIL ARE USED AS SAMPLES

Determinations made by direct-micrometer measurements.

(Each value is the average of four determinations for each size of sample.)

Weight of air-dried samples gm.	Weight of oven-dried samples gm.	Yolo clay loam		Volume of block of soil cc.	Apparent specific gravity
		Percentage of moisture retained	Thickness of block of soil cm.		
10.000	9.663	30.61	0.300	7.306	1.325±0.006
20.000	19.404	26.59	0.582	14.145	1.372±0.004
30.000	29.173	23.12	0.863	20.975	1.391±0.003
40.000	38.964	22.08	1.143	27.792	1.402±0.002
50.000	48.601	20.03	1.397	33.986	1.430±0.002
Oakley fine sand					
20.000	19.895	6.01	0.512	12.335	1.595±0.008
30.000	29.784	5.43	0.730	17.752	1.676±0.004
40.000	39.776	4.98	0.988	24.017	1.656±0.008

TABLE 11

APPARENT SPECIFIC GRAVITIES OF 30-GM. AIR-DRIED SAMPLES OF THE FIVE DIFFERENT SOILS AFTER CENTRIFUGING

Determinations made by direct-micrometer measurements.

(Each value is the average of four determinations for each size of sample.)

Soil used	Weight of oven-dried samples gm.	Percentage of moisture retained	Thickness of block of soil cm.	Volume of block of soil cc.	Apparent specific gravity
Yolo clay.....	28.580	30.01	0.893	21.702	1.317±0.005
Yolo silt loam.....	28.784	25.10	0.864	21.002	1.369±0.021
Yolo clay loam.....	29.173	23.12	0.863	20.962	1.391±0.003
Twin Falls silt loam....	28.990	22.31	0.840	20.430	1.419±0.001
Oakley fine sand.....	29.784	5.43	0.730	17.752	1.676±0.004

APPARENT SPECIFIC GRAVITIES OF SUCCESSIVE LAYERS  
OF SAMPLES

In order to determine the apparent specific gravity for successive thin layers of soil in a 60-gm. sample by direct-micrometer measurement of thickness, a special method of separating the several layers was devised as follows: Six 10-gm. samples of Yolo clay loam were accurately weighed. One 10-gm. sample was placed in the centrifuge cup on the filter paper which covered the wire gauze bottom. The surface was smoothed and levelled. A piece of thin paper cut accurately to the size of the cup was placed on top of the soil and another 10-gm. sample was placed on it. This process was continued until the cup contained the six 10-gm. layers or 60-gm. of air-dried soil. Each layer was separated by a sheet of thin paper. The thickness of the paper, as measured with a micrometer, was 0.038 mm. Two kinds of paper were used for separating the layers. In one series the paper partitions were porous or semi-porous. In the other series the paper was much less porous, being practically impervious.

After the cups were filled, the soil was moistened, centrifuged, and placed in a moist chamber in the usual manner. The measurements of the thickness of the blocks of soil constituting the successive layers were made in the same way as that just described for the single blocks of soil. Micrometer readings were made at the centers of the sixteen squares marked on the thin, flexible sheet of metal placed on the top of the soil of the last or innermost layer of the 60-gm. block. After these sixteen measurements were made, all of the soil in this layer was removed carefully and placed in a weighing bottle. The moisture content and the oven-dry weight of this soil were determined. The separating paper was removed from the surface of the next layer and the measurements of thickness repeated. The difference between the averages of the two sets of measurements gave the thickness of the layer plus the thickness of the separating paper. The thickness thus determined is corrected by multiplying the common factor, 1.0042, as previously explained. The measurement of the thickness of the last layer in the cup, or the layer farthest from the axis of rotation, does not include the thickness of separating paper. The soil of this layer was removed from the cup and the filter paper retained in the bottom of the cup. The average of the final micrometer readings

when corrected gave the average thickness of the bottom of the centrifuge cup and the filter paper which was subtracted from the average of the corrected micrometer readings made with the sheet metal piece on the top of the last layer of soil. The volumes of the layers were calculated in the manner previously described, and from these data the apparent specific gravity of each layer was calculated.

These apparent specific gravities of the 10-gm. layers of 60-gm. samples of Yolo clay loam after centrifuging are given in table 12. The percentage of moisture retained for the different layers are also given in the table. The values for the layers with porous paper separators are listed separately from those obtained for the layers of soil separated with the semi-impervious paper.

In some instances, the study of the distribution of moisture in the centrifuged blocks of soil reported in Section II give data from which the apparent specific gravities of the successive layers can be calculated.

The data reported in table 7 were obtained by slicing layers of centrifuged 60-gm. blocks of Yolo clay loam. These slices were made by the modified-microtome method in which blocks of soil were removed from the centrifuge cups without loss or breaking at the corners. Since the slices were made with precision, each slice being exactly 2 mm. thick, the volume of the soil in cubic centimeters in each slice can be accurately determined by multiplying the cross-section area of the block of soil (23.77 sq. cm.) by 0.2 cm. The number of grams of oven-dried soil in each slice divided by the volume gives the apparent specific gravity.

The apparent specific gravities of the successive layers of the blocks of soil for which the percentage of moisture retained are given in table 7, were calculated and are tabulated below.

Layer number	Grams of oven-dried soil in layers	Apparent specific gravity
1.....	6.371	1.304±0.007
2.....	6.477	1.362±0.009
3.....	6.877	1.436±0.012
4.....	6.837	1.438±0.014
5.....	7.119	1.498±0.015
6.....	6.836	1.438±0.013

Layer number 1 is nearest the axis of rotation. The apparent specific gravities of layers 7 and 8 are not tabulated on account of the uncertainty of the precision of slicing these last layers of the block of soil.

It will be noted that there is a significant increase in apparent specific gravity up to layer 3, and that beyond this it remains substantially constant.

TABLE 12

APPARENT SPECIFIC GRAVITIES AND PERCENTAGE OF MOISTURE RETAINED OF 10-GM. LAYERS WITH PAPER PARTITIONS OF 60-GM. SAMPLES OF YOLO CLAY LOAM  
(Layer No. 1 is nearest the axis of rotation.)

Layers with porous paper partitions	Number of layers.....	1	2	3	4	5	6
Trial number 1	Dry soil—gm.....	9.620	9.694	9.655	9.555	9.557	9.637
	Thickness—cm.....	0.295	0.288	0.255	0.286	0.275	0.263
	Volume—cc.....	7.127	6.958	6.160	6.910	6.644	6.354
	Apparent specific gravity.....	1.35	1.39	1.57	1.38	1.44	1.52
	Percentage of moisture retained.....	20.81	20.77	22.19	21.95	21.15	20.10
Trial number 2	Dry soil—gm.....	9.673	9.645	9.652	9.650	9.656	9.636
	Thickness—cm.....	0.298	0.283	0.273	0.280	0.273	0.265
	Volume—cc.....	7.200	6.837	6.596	6.765	6.597	6.403
	Apparent specific gravity.....	1.34	1.41	1.46	1.43	1.46	1.50
	Percentage of moisture retained.....	21.02	21.51	22.36	22.06	21.51	21.30
Trial number 3	Dry soil—gm.....	9.560	9.665	9.657	9.645	9.583	9.626
	Thickness—cm.....	0.293	0.293	0.265	0.272	0.267	0.283
	Volume—cc.....	7.079	7.079	6.403	6.572	6.451	6.837
	Apparent specific gravity.....	1.35	1.36	1.51	1.47	1.48	1.41
	Percentage of moisture retained.....	21.08	21.41	21.63	22.18	21.80	21.22
Means.....	Apparent specific gravity.....	1.35	1.39	1.51	1.43	1.46	1.48
	Percentage of moisture retained.....	20.97	21.23	22.06	22.06	21.49	20.86

TABLE 12—(Continued)

APPARENT SPECIFIC GRAVITIES AND PERCENTAGE OF MOISTURE RETAINED OF 10-GM.  
LAYERS WITH PAPER PARTITIONS OF 60-GM. SAMPLES OF YOLO CLAY LOAM  
(Layer No. 1 is nearest the axis of rotation.)

Layers with semi-impervious partitions	Number of layer.....	1	2	3	4	5	6
Trial number 4	Dry soil—gm.....	9.569	9.618	9.746	9.639	9.649	9.619
	Thickness—cm.....	0.320	0.294	0.289	0.261	0.279	0.280
	Volume—cc.....	7.731	7.103	6.982	6.306	6.741	6.765
	Apparent specific gravity.....	1.24	1.35	1.39	1.53	1.43	1.42
	Percentage of moisture re- tained.....	31.36	28.31	28.95	28.19	25.51	23.58
Trial number 5	Dry soil—gm.....	9.649	9.596	9.602	9.604	9.649	9.644
	Thickness—cm.....	0.304	0.327	0.264	0.271	0.274	0.275
	Volume—cc.....	7.314	7.900	6.378	6.547	6.620	6.644
	Apparent specific gravity.....	1.31	1.21	1.51	1.47	1.46	1.45
	Percentage of moisture re- tained.....	36.49	32.76	31.07	29.42	26.93	24.94
Trial number 6	Dry soil—gm.....	9.546	9.597	9.621	9.582	9.653	9.607
	Thickness—cm.....	0.325	0.292	0.276	0.287	0.269	0.275
	Volume—cc.....	7.852	7.055	6.668	6.934	6.499	6.644
	Apparent specific gravity.....	1.22	1.36	1.44	1.38	1.48	1.45
	Percentage of moisture re- tained.....	34.97	32.50	29.28	29.35	27.35	24.95
Means.....	Apparent specific gravity.....	1.26	1.31	1.45	1.46	1.46	1.44
	Percentage of moisture re- tained.....	34.27	31.19	29.77	28.99	26.60	24.49



## SECTION IV

## INFLUENCE OF ARTIFICIAL PACKING

When 30 gm. of soil are placed in the centrifuge cup and subjected to a centrifugal force of 1000 times gravity, there is developed a pressure of probably 30,000 gm. on the filter paper at the bottom of the cup. In order to determine whether this pressure in itself has any effect upon the moisture retained by the soil, artificial means of applying pressure of different magnitude to different amounts of soil were tried.

## COMPACTING BY SUPERIMPOSED WEIGHTS OF SOIL

A preliminary test was made to determine whether this factor was large enough to warrant further study.

In the first part of this test, 10 gm. of soil were placed in each of twelve cups, moistened, and allowed to stand twenty-four hours. Just before centrifuging, a double layer of paraffined paper was placed on top of each 10-gm. sample.

Successively, in duplicate, 10, 20, 30, 40, and 50 gm. of soil, slightly moistened, were placed on the paraffined paper, leaving the remaining two cups as check samples. The soil used as a weighting substance was moistened enough to remain in position on edge until the centrifugal force would hold it in position. After centrifuging, the soil layers used as weights were removed and weighed, and moisture determinations were made on the 10-gm. samples below in the usual way by transferring the moist soil sample to a soil can and discarding the filter paper. Similarly, ten cups were filled with 20 gm. of soil and just before centrifuging were weighed in duplicate by 10, 20, 30, and 40 gm. of moistened soil, leaving the remaining two cups as checks. Again, eight cups were filled with 30 gm. of soil and weighted in duplicate with 10, 20, and 30 gm. with two cups not weighed. Table 13 gives the results of this test.

The difference between each observation and the mean in no case exceeded one-half of one per cent. The tests wherein the difference between observations and the mean exceeds one-fourth of one per cent of moisture are marked with an asterisk.

TABLE 13

RESULTS OF PRELIMINARY TEST ON THE INFLUENCE OF VARYING PRESSURES ON THE PERCENTAGE OF MOISTURE RETAINED BY 10, 20, AND 30 GM. OF AIR-DRIED YOLO SILT LOAM WHEN SUBJECTED TO A CENTRIFUGAL FORCE OF 1000 TIMES GRAVITY

Number of trials	Weight of soil used as weighting material including its moisture <i>gm.</i>	Weight of oven-dry soil <i>gm.</i>	Percentage of moisture retained
2.....	0	9.72	30.1
2.....	10.75	9.75	28.2*
2.....	21.10	9.72	26.2*
2.....	31.15	9.85	25.1
2.....	41.30	9.85	23.9*
2.....	51.10	9.68	23.3
2.....	0	19.78	27.8*
2.....	10.50	19.62	27.25
2.....	20.40	19.85	26.2
2.....	31.30	19.68	25.2
2.....	41.30	19.68	24.5
2.....	0	29.95	25.2
2.....	10.95	29.90	24.9*
2.....	20.60	29.90	24.5*
2.....	31.10	29.80	24.3

Because of lack of sufficient replications these data alone are not conclusive, but they suggest that an increase in the pressure on the soil during centrifuging causes a decrease in moisture which the soil can retain against the centrifugal force of 1000 times gravity.

Certain objections to the use of soils as the weighting material presented themselves:

1. Probably because of the fact that the paraffine paper did not fit tightly into the corners of the centrifuge cups, the soil used as a weight did not exert the same pressure over the whole of the upper surface of the soil in the bottom of the cup.

2. The weighting soil after centrifuging was found to be heavier than it was when it was placed on the paraffined paper before centrifuging. Some moisture might have been taken up by capillarity even through the paraffined paper.

COMPACTING BY SUPERIMPOSED IRON SQUARES

To obviate these difficulties, all subsequent trials were made with squares of galvanized sheet-iron as the weighting material. These were cut so that they would leave approximately 0.1 mm. between the square and the side of the cup. The squares of sheet-iron were bent to conform as nearly as possible to the curve of the bottom of the cup. The weight on top of each sample of soil in the cup was secured by adding enough squares to equal the weight desired.

The effect of using the galvanized iron squares as weights on 5 gm. of air-dry Yolo silt loam was next observed. Table 14 gives these results as the averages of four observations on each trial.

TABLE 14

RESULTS OF TESTS ON THE INFLUENCE OF ARTIFICIAL WEIGHTING ON THE PERCENTAGE OF MOISTURE RETAINED BY APPROXIMATELY 5 GM. OF AIR-DRIED YOLO SILT LOAM AFTER CENTRIFUGING

Number of trials	Weight of sheet-iron squares gm.	Weight of oven-dry soil gm.	Percentage of moisture retained
4.....	0	4.82	36.17±0.00
4.....	12.25	4.86	33.86±0.14
4.....	24.95	4.86	32.21±0.16
4.....	30.80	4.85	31.63±0.28

It will be observed that, as suggested before, an increase in the magnitude of the compressing force decreases the percentage of moisture retained against the centrifugal force of 1000 times gravity.

The weight of a 30-gm. air-dry sample of Yolo silt loam soil and the moisture which it normally retains after centrifuging is a little over 37 gm. The possible distribution of moisture in the soil after centrifuging is suggested by the following series of trials, made with Yolo silt loam. As the amount of soil was increased, the weight of the sheet-metal squares was reduced so that the sum of the moist soil and the metal squares would equal approximately 37 gm. Table 15 gives the results of these trials.

Most of the intensive work done in other phases of this subject has been with Yolo clay loam. For the purpose of obtaining some data on this soil, 10-gm. samples were subjected to different compres-

sion forces due to varying the weight of metal squares used in each case. Table 16 gives results for the average of four in each trial for these observations.

TABLE 15

INFLUENCE OF ARTIFICIAL WEIGHTING ON THE PERCENTAGE OF MOISTURE RETAINED AFTER CENTRIFUGING, BY INCREASING AMOUNTS OF YOLO SILT LOAM AND DECREASING WEIGHT OF SHEET-IRON SQUARES

Number of trials	Weight of sheet-iron squares, in grams	Weight of oven-dry soil, in grams	Percentage of moisture retained
4.....	0	29.55	26.44±0.08
4.....	6.05	24.58	27.32±0.05
4.....	12.40	19.70	27.88±0.10
4.....	18.70	14.73	27.76±0.09
4.....	24.60	9.70	28.82±0.20
4.....	30.80	4.85	31.63±0.28

TABLE 16

INFLUENCE OF ARTIFICIAL WEIGHTING ON DIFFERENT MAGNITUDES ON THE PERCENTAGE OF MOISTURE RETAINED AFTER CENTRIFUGING OF APPROXIMATELY 10 GM. OF YOLO CLAY LOAM

Number of trials	Weight of iron squares gm.	Weight of oven-dry soil gm.	Percentage of moisture retained
12.....	0	9.69	32.22±0.11
4.....	12.5	9.76	30.80±0.08
4.....	24.1	9.79	29.56±0.08
4.....	37.1	9.64	28.67±0.31
4.....	48.8	9.62	27.58±0.19
4.....	73.9	9.63	27.32±0.05
4.....	86.1	9.80	26.46±0.24
4.....	147.7	9.62	26.46±0.21

It will be noted that with the exception of the trials with 73.9 gm. and 147.7 gm. of iron squares as superimposed weights, there are significant decreases in the successive percentage of moistures retained as the superimposed weight is increased.

## SECTION V

## DISCUSSION OF OBSERVATIONS

The experimental observations reported above indicate, among other things, the existence of the four following conditions:

1. That the use of the smaller samples of soil results in relatively large percentages of moisture, and that the rate of decrease in the percentage of moisture retained with increase in size of sample is higher for small samples than for large ones.

2. That within a 60-gm. sample of soil the percentage of moisture increases from the inner to the outer surface, but that the rate of increase is appreciably lower than that which is found by reducing the size of sample.

3. That the apparent specific gravity,  $A_s$ , of a 60-gm. sample after centrifuging, is significantly greater than that of a 10-gm. sample, and as the size of the sample is decreased from a 60 to a 10-gm. sample,  $A_s$  decreases continuously; also, that within a 60-gm. sample,  $A_s$  increases significantly outward for about 6 mm., beyond which it remains approximately constant to the outer surface.

4. That with 5, 10, 20, or 30-gm. samples, increasing the pressure on the soil, by placing extra weights on the inner surface, decreases significantly the amount of water retained by the soil.

## SYMBOLS USED

The following symbols are used in the discussion. (Other symbols also are used, but these are specifically defined in each instance.)

$S$  = Percentage of pore space based on total volume.

$P_v$  = Percentage of the total volume occupied by water, or grams of water per cubic centimeter of soil.

$P_w$  = Percentage of water based on water-free weight of soil.

$A_s$  = Apparent specific gravity or volume weight.

$R_s$  = Real specific gravity of the soil.



## EXPLANATION ADVANCED BY PREVIOUS INVESTIGATIONS

The fact that the percentage of moisture retained against the centrifugal force decreases as the thickness of the soil sample increases has been observed by Thomas<sup>12</sup> and by Joseph and Martin.<sup>9</sup> But so far as we are aware, the distribution of moisture within the sample of soil has not heretofore been investigated. As previously mentioned, Thomas<sup>12</sup> proposed a "partial solution of the problem." He equated the capillary-potential gradient to the normal component of the centrifugal force for unit mass thus:

$$\frac{d\psi}{dr} = + r\omega^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

Integrating (1) and letting  $\psi_c$  equal the potential at the "center of the centrifuge," that is the potential when  $r=0$ , Thomas<sup>12</sup> finds

$$\psi = \frac{r^2\omega^2}{2} + \psi_c \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Having found experimentally that a 5-gm. layer of soil contained 31.7 per cent of water he concluded that the outer 5-gm. layer of the 70-gm. block would also contain 31.7 per cent. Our study of the distribution of moisture within a 60-gm. block, however, indicates that the outer 2-mm. layer contains much less moisture than that held by a single 2-mm. block. For example, table 6 shows that the outer 2-mm. layer of a 60-gm. sample contained only 2.58 per cent more than the inner 2-mm. layer, whereas the 5-gm. sample of Yolo clay loam reported in table 1 held 22.60 per cent more than the 60-gm. sample. Table 7 shows that the outer 4-mm. layer of a 60-gm. block contained only 2.38 per cent of moisture more than the inner 2-mm. layer.

THEORETICAL DISCUSSION OF AN EQUIPOTENTIAL REGION  
THROUGHOUT THE BLOCK OF CENTRIFUGED SOIL  
AND ITS APPLICATION

The use of energy relations is of value in a further solution of the problem. A review of well-known energy conditions under a familiar physical state is helpful toward visualizing the distribution of energy of different forms within the soil blocks. It is common knowledge that the surface of a small pond or lake is level if undisturbed by

wind or other extraneous forces. If a plane\* parallel to the surface of the lake, which passes through a point 0 at a depth  $D$  below the lake surface be selected as the datum, it is clear that the gravitational potential energy of unit mass in the lake surface, with respect to the datum plane, is everywhere equal. Using cartesian coördinates, let the  $X$  and  $Y$  axes lie in the  $D$  plane of the paper as shown in figure 8a, and let the  $Z$  axis  $D$  lie in the datum plane at right angles to the plane of the paper.

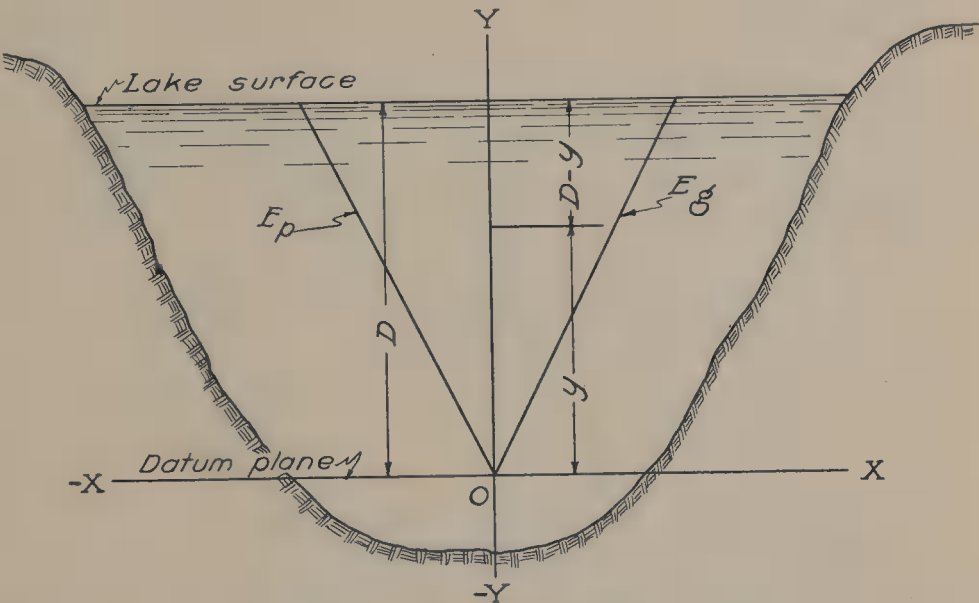


Fig. 8a. Relation between the gravitational potential energy and the hydrostatic-pressure energy of unit mass of water at any point in a lake with reference to a plane at a depth  $D$  below the lake surface.

Let  $E_g$  = the gravitational potential with respect to the  $X$ - $Z$  plane at any point  $y$ , and

Let  $E_p$  = the hydrostatic-pressure potential with respect to the  $X$ - $Z$  plane at any point  $y$ .

If the influence of hydrostatic pressure be ignored, the work in gram centimeters required to lift unit mass of water from the  $X$ - $Z$  plane, in which  $y = 0$ , to any point a distance  $y$  above the plane, is

$$E_g = \int_0^y dy = [y]_0^y = y \quad \dots \dots \dots (3)$$

At any point distant  $y$  above datum, the hydrostatic pressure in gm. per sq. cm. is  $P = (D - y)$ .

\* Since only a small area is involved, the surface of the lake, or any surface parallel thereto is considered to be a plane.

It is evident from the equation for the value of  $P$ , that  $P$  is a maximum when  $y = 0$  and that  $P = 0$  when  $y = D$ . As  $y$  increases  $P$  clearly decreases, hence the resultant force  $F_p$ , due to change in  $P$  with change in  $y$ , is directed upward. This shows that if the direct influence of the force of gravity were removed, the difference in pressures would tend to cause movement upward. In other words, the work required to move unit mass of water from 0 to  $y$  against the force due to change of pressure is negative. The magnitude of the force  $F_p$  is obtained by differentiating  $P$  with respect to  $y$ , thus:

$$F_p = \frac{dP}{dy} = \frac{d}{dy}(D - y) = -\frac{dy}{dy} = -1,$$

and hence the work required, neglecting the influence of gravity, to lift unit mass against the hydrostatic pressure is

$$E_p = -\int_0^y dy = -y \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Adding equations (3) and (4), it is found that the total energy is

$$E_g + E_p = y + (-y) = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

The lake surface corresponds to the inner surface of the soil block in the centrifuge cup, and the datum plane, at a depth,  $D$ , in the lake, corresponds to the outer surface of the soil block. Moving unit mass of water from the outer surface of the soil block to the inner surface is analogous to moving unit mass from the datum plane in the lake to the lake surface. Passing through the soil block along the line of the radius of the centrifuge, the total energy of unit mass of water is everywhere the same, if the moisture is in equilibrium; but, as in the lake, either form of energy increases as the other decreases.

The energy density at a point in the water within the soil block is largely of two kinds, namely:

(1) "Centrifugal" field energy density which is numerically equal to the work required to move unit mass from the outer surface inward, against the "centrifugal" force of the rotating machine, and

(2) Capillary energy density, or energy density due to surface curvature, which is numerically equal to the work required to move unit mass against the capillary forces from the surface of free water (which may be a real surface within the soil block or an imaginary

surface outside the soil block) to the point in question.\* This work according to the reference base used by Buckingham<sup>5</sup> is the capillary potential.

The capillary potential thus defined is negative because the direction of the resultant capillary force is inward; opposite to the direction of the "centrifugal" force. Gardner<sup>6</sup> says, "It is perhaps quite immaterial where the zero potential is placed." However, for measurements of capillary potential which have not yet been published, Gardner also has selected the water table as the surface of zero potential.

If the soil in the outer surface of the centrifuged soil block is saturated, and if in this surface the water is neither under tension nor compression stresses, then this outer surface would be one of zero capillary potential according to the definitions given by Buckingham and by Gardner. Our study of the percentage of soil pore space occupied by water reported in figure 9 and discussed therewith, suggests that for the particular soil used, the outer surface of the soil block and the surface of zero potential may be in close proximity. However, in the absence of definite information concerning this point, or concerning the influence of the boundary conditions of the outer surface, it is important to keep in mind the fact that the capillary potential,  $\psi$ , as here used does not include the work required to move unit mass from the free water surface to the outer surface of the soil block. To arrive at the magnitude of  $\psi$  with reference to a free water surface we must include the work required to move unit mass from such surface, which is the surface of zero capillary potential, to the outer surface of the soil block. This work will vary for different soils and is undetermined. However, as we are here concerned primarily with the space rate of change of the capillary potential within the soil

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\* That the energy density is numerically equal to the capillary potential is apparent from the following:

Let  $e$  = the energy density, or energy divided by volume.

$dv$  = differential volume.

$\psi$  = the capillary potential, or work divided by mass.

$dm$  = differential mass.

$\rho$  = moisture density or grams water per c.c. volume.

Then  $e dv = \psi dm$ . But  $dm = \rho dv$

therefore,

$$e dv = \psi \rho dv \quad \text{and} \quad e = \rho \psi.$$

Since, with the units we use, one gram of water has unit volume, we have

$$\rho = 1$$

and  $e = \psi$  numerically.

block, we present the following analysis despite our lack of knowledge concerning the position of the surface of zero capillary potential.

The properties of the above two forms of energy may be clearly understood by reference to figure 8b. The origin of coördinates is taken at a point in the outer surface of the soil block. The  $X$ - $Z$  plane is here made tangent to the outer surface of the soil, and the  $X$ - $Y$  plane intersects the soil block at right angles, and passes through the axis of rotation.

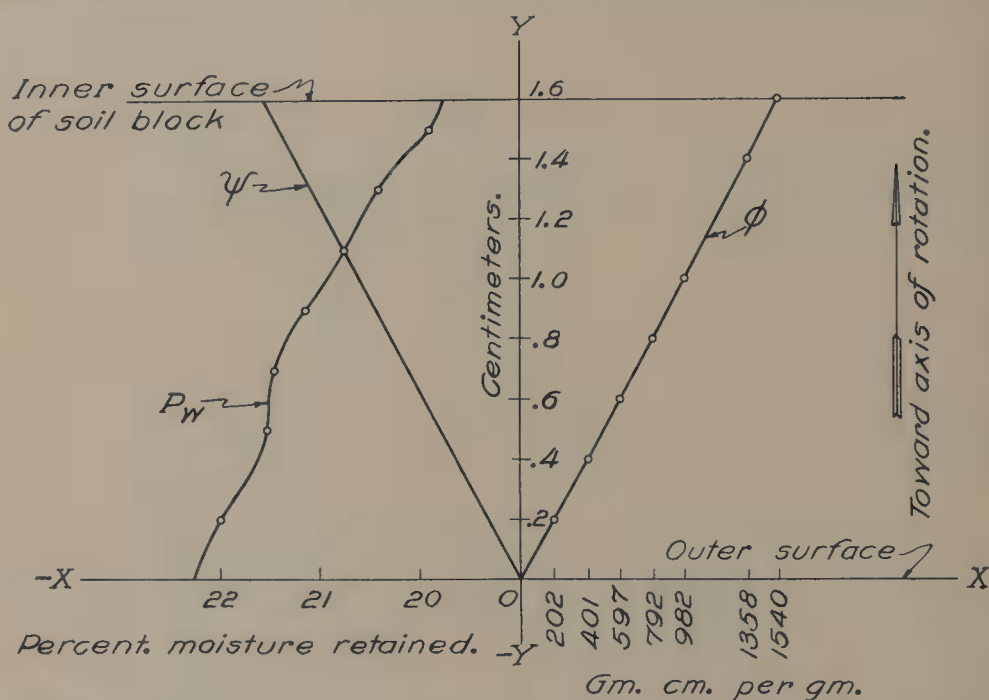


Fig. 8b. Relation between the "centrifugal" potential energy and the capillary potential energy in the soil blocks with respect to the outer surface of soil.

This arrangement of coördinates clearly places the  $X$ - $Z$  plane at right angles to the direction of the "centrifugal" force, it being thus similar to its position with respect to the force of gravity in figure 8a.

Let  $\phi$  = the "centrifugal" field potential (analogous to  $E_g$ ) with respect to the  $X$ - $Z$  plane.

Let  $\psi$  = the capillary-pressure potential (analogous to  $E_p$ ) with respect to the  $X$ - $Z$  plane.

If the influence of the capillary-pressure force be ignored, the work required to move unit mass from the  $X$ - $Z$  plane in which  $y=0$  to any point a distance  $y$  from the plane is equal to  $\phi$ , the "centrifugal" field potential.



The magnitude of the "centrifugal" force at any point  $y$  is  $F_c = \omega^2(R - y)$  where  $\omega$  is the angular velocity. Hence, the work on unit mass against this force for a given velocity is

$$\phi = \omega^2 \int_0^y (R - y) dy = \omega^2 \left[ \int_0^y R dy - \int_0^y y dy \right]$$

and, therefore, 
$$\phi = \omega^2 y \left( R - \frac{y}{2} \right) \dots \dots \dots (3')$$

If it were possible to write the value of the capillary-pressure potential,  $\psi$  at any point distant  $y$  from the origin, as it is to write the value of the hydrostatic-pressure potential  $E_p$  (see eq. 4), it could at once be proved that  $\phi = -\psi$  in the same way as it was proved above that  $E = -E_p$ . Reasoning by analogy, however, it is apparent that if the system is in equilibrium, then as the value of  $y$  varies from 0 to the thickness of the soil block, the negative capillary potential,  $\psi$ , must increase at the same rate as does the positive "centrifugal" potential,  $\phi$ .

If  $\psi$  has thus been determined, and the moisture percentages,  $P_w$ , which determines the particular values of  $\psi$  at different points are also determined by the laboratory methods described above, it may be possible to arrive at a relation between the capillary-pressure potential and the moisture content, namely:

$$\psi = f(P_w)$$

which is clearly analogous to the relation of the hydrostatic-pressure potential and the depth of the water in the lake, namely:

$$E_p = f(y) = -y.$$

The application of the reasoning above is illustrated by the following examples. In equation (3), let  $y = D = 1000$  cm. The force of gravity on unit mass is 1 gm. Lifting this unit mass against a force of 1 gm. through a distance of 1000 cm. requires the doing of 1000 gm.-cm. of work and thus stores 1000 gm.-cm. of gravitational potential energy in the unit mass. At the same time, hydrostatic-pressure energy decreases from  $E_p = 0$  at the origin to  $E_p = -1000$  gm.-cm. when  $y = D$ .

Now, if equation (3') be used to compute the work done against the "centrifugal" force,  $F_c$ , it is seen that since  $\omega = \frac{2\pi N}{60}$ , where  $N = 2440$ , the speed at which the centrifuge was run, and hence

$$\omega^2 = \left( \frac{2\pi 2440}{60} \right)^2 = 65290,$$

therefore  $\phi = 65290 y \left( 15.24 - \frac{y}{2} \right)$  dyne centimeters.

Below are given some values of  $\phi$  for different values of  $y$ , as computed from the above equation.

Distance from outer surface' $y$	Work done against the "centrifugal" force, $\phi$	
	dyne-cm.	gm.-cm.
0.2	197700	202
0.4	392800	401
0.6	585200	597
0.8	775100	792
1.0	962400	982
1.4	1329000	1358
1.6	1508000	1540

The tabulation above shows that moving a gram of water from the outer surface of the soil block toward the axis of rotation a distance of 1 cm. against the "centrifugal" force, requires 982 gm.-cm. of work, or an amount equal to that required to lift one gram a vertical distance of 982 cm. against the force of gravity.

These values of  $\phi$  for different values of  $y$  are plotted in figure 8b. Although equation (3') is not the equation of a straight line, yet figure 8b shows, since the value of  $y$  is small as compared to  $\omega$  and  $R$ , the curve of  $\phi$ , within the limits of  $y$  considered, appears to be a straight line.

Figure 8b also shows that as  $y$  increases and the "centrifugal" field-energy is increased, the capillary-pressure potential must increase; and since  $\psi$  is negative, its absolute value decreases as its algebraical value increases. This shows, moreover, that at the points where  $\psi$  is numerically large, the moisture content  $P_w$  must be small, and where  $\psi$  is numerically small,  $P_w$  must be large.

In figure 8b the  $X$  axis also represents percentage of moisture. The origin is taken at the point of 19 per cent of moisture. The moisture increases toward the left of the origin. The small circles plotted to the left of the  $Y$  axis represent the observed moisture percentages reported in table 7, and plotted in figure 4.

It seems reasonable to believe that the capillary-potential gradient is a measure of the force resisting the "centrifugal" force, or vice versa, and therefore, that the adhesive forces between soil and water are negligible for moisture percentages equal to or greater than those in the centrifuge cups. It therefore seems reasonable to expect an approximately uniform rate of change of moisture content through the soil block. This implies that the relation between  $\psi$  and  $P_w$ , not yet definitely established, is approximately linear.

Figure 4 and table 7 indicate that in the second to fifth layers of soil the rate of increase is nearly constant; that it decreases through the sixth and seventh layers and again increases through the eighth or outside layer. The form of the curve in figure 4, and more particularly the form of the two curves in figure 7 suggest that final equilibrium had not been reached and that a longer period of operation of the centrifuge might have brought about a more nearly uniform rate of increase of moisture content with increase in radius.

It is clearly desirable, as pointed out by Buckingham<sup>5</sup> and by Gardner,<sup>6</sup> to find how the capillary potential depends on the moisture content of the soil. Studying theoretically the distribution of moisture in vertical soil columns when in equilibrium with the force of gravity, Buckingham<sup>5</sup> found from the analysis of energy relations that the rate of change of  $\psi$  with change in vertical position  $x$  was constant; that is,

$$\frac{\delta\psi}{\delta x} = \frac{9}{100} = A = \text{constant} \quad . . . . . (5)$$

As the lower end of his experimental soil columns were in contact with gravitational water, and as  $\psi$  is zero at the surface of the water where the soil is saturated and where  $x$  is zero, he found under these conditions that  $\psi = Ax$ . He determined the moisture content,  $P_w$ ,\* at different points in several soil columns which were about 50 inches high. Despite the fact that some of these columns were kept in contact with water for nearly a year, uncertainty exists as to whether the moisture had reached final equilibrium. Several difficulties arise in the use of this method of studying the relation of  $\psi$  to  $P_w$ ; namely, the length of time required to establish equilibrium by keeping the soil columns in direct contact with water, the maintenance of a constant temperature, and the handling of long soil tubes.

\* Buckingham<sup>5</sup> used the symbol  $\Theta$  to represent moisture content.

A thorough study of the relation between the capillary potential,  $\psi$ , and the moisture content,  $P_w$ , is beyond the scope of this paper.

In a particular soil of a given moisture content, the capillary potential is clearly influenced by the degree of compactness and the compactness is reflected in the apparent specific gravity ( $A_s$ ).

The significance of our measurements of  $A_s$  after centrifuging and some possible relations to  $P_w$ , upon which  $\psi$  depends in part, are considered below.

#### THE RELATIVE SATURATION OF CENTRIFUGED SAMPLES

Since by definition the

$$\text{Pore space} = \frac{R_s - A_s}{R_s}$$

Therefore, the per cent pore space by volume is

$$S = 100 \left[ 1 - \frac{A_s}{R_s} \right]$$

The percentage of water on a volume basis,  $P_v$ , cannot exceed the percentage of pore space, hence  $\frac{P_v}{S}$  must always be less than unity, unless the soil is saturated. It may be shown as follows that

$$P_v = A_s P_w.$$

For example, consider one liter of oven-dried soil which weighs 1300 gms. to which 150 cc. of water has been added.

$$\text{Then, } P_v = \frac{150 \times 100}{1000} = 15$$

$$P_w = \frac{150 \times 100}{1300} = \frac{150}{13}$$

$$\text{and } A_s = \frac{1300}{1000} = 1.3$$

$$\text{Therefore, } P_v = 1.3 \times \frac{150}{13} = 15.$$

The  $P_w$ ,  $P_v$ ,  $A_s$ , and  $\frac{P_v}{S}$  for all of the samples for which  $A_s$  were determined after centrifuging are listed in tables 17 and 18.

In table 17 the percentages of pore spaces occupied by water is given for the whole centrifuged sample. The calculated value represents the average percentage of the pore space filled with water, or

TABLE 17

PERCENTAGE OF THE PORE SPACE OCCUPIED BY WATER IN CENTRIFUGED SAMPLES  
OF DIFFERENT SOILS AND OF DIFFERENT WEIGHTS  
(The  $\frac{P_v}{S}$  of the whole sample given in this table.)

Soil based	Air-dried samples gm.	Weight of oven-dry soil gm.	Percentage of moisture retained		Specific gravities		S	$\frac{P_v}{S}$	Original data reported
			$P_w$	$P_v$	$A_s$	$R_s$			
Yolo clay loam.....	10.000	9.726	31.80	36.79	1.16	2.685	56.85	55.5	Table 9.
Yolo clay loam.....	20.000	19.452	26.86	33.84	1.26	2.685	53.13	63.8	"
Yolo clay loam.....	30.000	29.178	24.66	33.29	1.35	2.685	49.88	66.7	"
Yolo clay loam.....	60.000	58.356	21.71	31.26	1.44	2.685	46.43	67.4	"
Yolo clay loam.....	10.000	9.663	30.61	40.58	1.32	2.685	50.65	80.1	Table 10.
Yolo clay loam.....	20.000	19.404	26.59	36.42	1.37	2.685	48.90	74.5	"
Yolo clay loam.....	30.000	29.173	23.12	32.15	1.39	2.685	48.20	66.7	"
Yolo clay loam.....	40.000	38.964	22.08	30.93	1.40	2.685	47.80	64.7	"
Yolo clay loam.....	50.000	48.601	20.03	28.63	1.43	2.685	46.77	61.2	"
Oakley fine sand.....	20.000	19.895	6.01	9.99	1.50	2.663	41.39	23.2	"
Oakley fine sand.....	30.000	29.784	5.43	9.10	1.68	2.663	38.39	23.7	"
Oakley fine sand.....	40.000	39.776	4.98	8.24	1.66	2.663	39.12	21.1	"
Yolo clay.....	30.000	28.580	30.01	39.52	1.32	2.724	51.65	76.6	Table 11.
Yolo loam.....	30.000	28.784	25.10	34.35	1.37	2.718	49.61	69.3	"
Twin Falls silt loam.....	30.000	28.990	22.31	31.69	1.42	2.705	47.53	66.6	"

(Each value is the average of four determinations.)

TABLE 18

PERCENTAGE OF THE PORE SPACE,  $\frac{P_v}{S}$  OCCUPIED BY WATER IN SUCCESSIVE LAYERS  
OF CENTRIFUGED YOLO CLAY LOAM SAMPLES  
(Layer No. 1 is nearest axis of rotation.)

	Layer number	Weight of oven-dried soil in samples gm.	Percentage of moisture retained		Specific gravities		S	$\frac{P_v}{S}$	Original data reported
			$P_w$	$P_v$	$A_s$	$R_s$			
10-gm. layer of a 60-gm. block of soil with porous paper partitions.*	1	9.618	20.97	28.31	1.35	2.685	49.70	57.0	Table 12.
	2	9.668	21.23	29.51	1.39	"	48.21	61.2	"
	3	9.654	22.06	33.29	1.51	"	43.78	76.1	"
	4	9.683	22.06	31.54	1.43	"	46.72	67.6	"
	5	9.598	21.49	31.35	1.46	"	45.60	68.7	"
	6	9.633	20.86	30.85	1.48	"	44.85	68.8	"
10-gm. layer of a 60-gm. block of soil with semi-imperious paper partitions.	1	9.588	34.27	43.19	1.26	"	53.35	81.0	"
	2	9.604	31.19	40.85	1.31	"	51.50	79.3	"
	3	9.656	29.77	43.15	1.45	"	45.98	93.9	"
	4	9.608	28.99	42.31	1.56	"	45.60	92.7	"
	5	9.650	26.60	38.85	1.46	"	45.60	85.2	"
	6	9.623	24.49	35.25	1.44	"	46.32	76.1	"
2-mm. slices from 60-gm. block of soil.†	1	6.371	19.66	26.18	1.34	"	50.40	56.9	Table 7.
	2	6.477	20.35	27.55	1.36	"	49.59	56.9	"
	3	6.877	20.76	29.61	1.44	"	46.83	63.3	"
	4	6.837	21.19	30.30	1.44	"	46.75	64.8	"
	5	7.119	21.59	32.13	1.50	"	44.58	72.1	"
	6	6.836	21.75	31.10	1.44	"	46.75	66.6	"

\*Each value is the mean of three determinations.

†Each value is the mean of eight determinations.



probably the value near the center of the block of soil constituting the sample. These data are based upon the determination of  $A_s$  by the paraffine-immersion method and the direct-micrometer measurement method on the entire samples as described on pages 30 and 31. In table 18 similar data are given for the successive layers of the centrifuged samples. For these,  $A_s$  was determined by direct-micrometer measurements. The  $A_s$  of the 2-mm. slices of the 60-gm. blocks of soil made by means of the improved microtome method are also given in the table 18. The ratio of the percentage of water on the volume basis,  $P_v$  to  $S$ , in this case is likewise the average for the slice, and may be taken as the value near the center of the slice.

From an examination of table 17, it will be noted that the average value of  $\frac{P_v}{S}$  for the entire centrifuged samples indicates that the amount of water retained against the centrifugal force is not limited by the pore space. This conclusion, with two possible exceptions, seems also to be confirmed by examination of the data presented in table 18. The two exceptions are the layers numbers 3 and 4 of the 60-gm. block of soil, which were separated by semi-impervious paper partitions, and which consequently were not in intimate capillary contact.

The data for the 10 to 50-gm. blocks of Yolo clay loam, from which  $A_s$  was determined by direct-micrometer measurements originally reported in table 10, suggest that the outer layers of a 60-gm. block of soil might be saturated, as do also the data for the 2-mm. slices cut from the 60-gm. blocks of soil by the improved microtome method.

Figure 9 graphically illustrates the increase in ratio of  $P_v$  to  $S$ , as we go from the inner surface of the block of soil to the outer surface. The calculated  $\frac{P_v}{S}$  for layers 6, 7, and 8 are not plotted because of unreliability of the determination of  $A_s$  for these slices. As previously pointed out, it was extremely difficult to cut accurately the last two slices from the block of soil. Since the third from the last layer, number 6, was somewhat difficult to slice, it also is not plotted. The curve of figure 9 for the first five slices of the block of soil is approximately a straight line, and prolonging this indicates that  $\frac{P_v}{S}$  at the outermost layer of soil is 90 per cent. It is not unreasonable, then, to suppose that while the extreme outer layers of a centrifuged

block of Yolo clay loam may be saturated, the limit of the amount of water retained by the soil against the centrifugal force is not determined by the pore space of the entire block of soil.

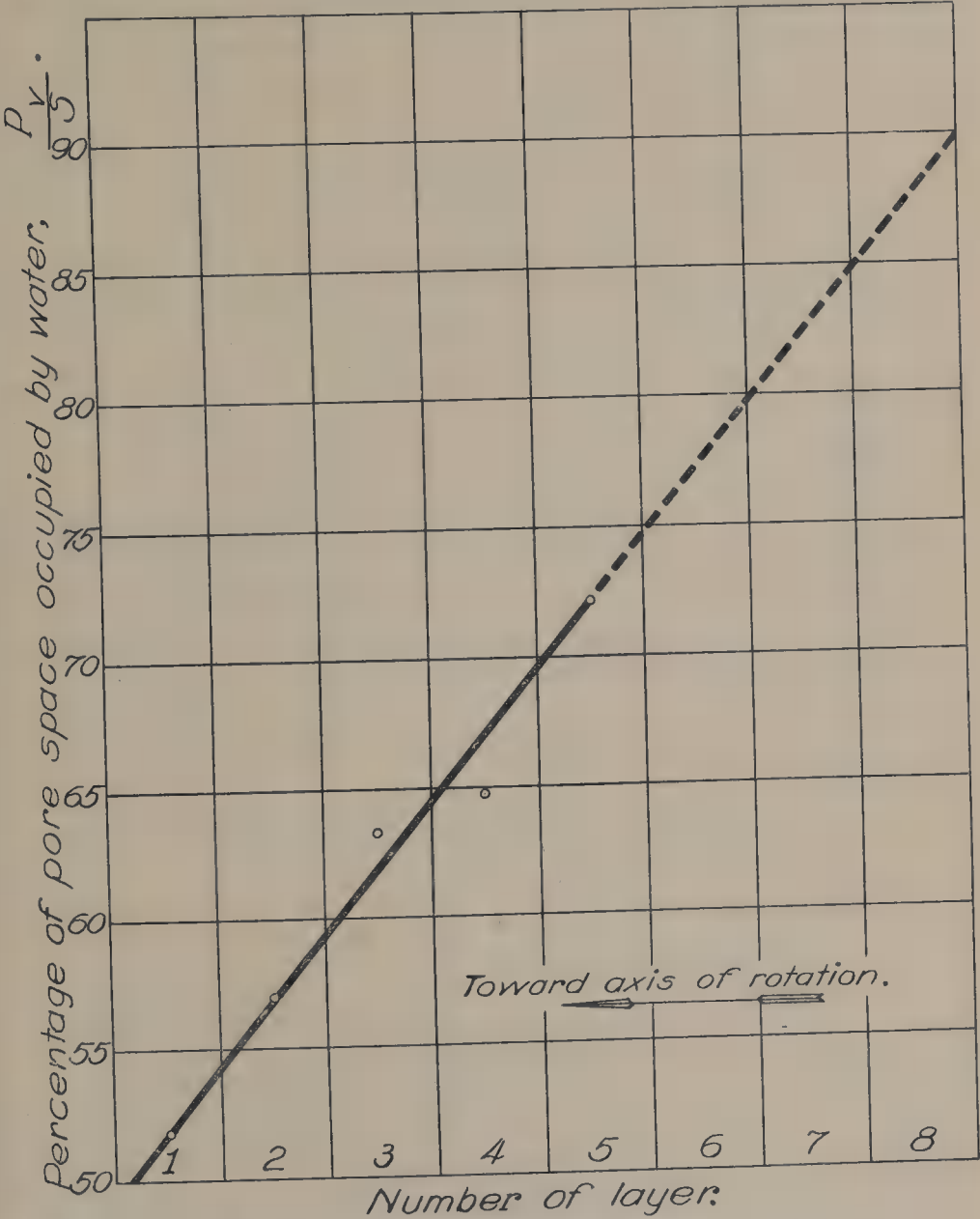


Fig. 9. Percentage of pore space occupied by water in consecutive layers of a 60-gm. block of Yolo clay loam soil after centrifuging.

The readjustment of moisture in the layers of the centrifuged blocks of soil when the centrifuge machine was being stopped may have been rapid enough to affect the percentage of moisture found in these

layers at the time they were sliced. The distribution of moisture within 60-gm. samples of Yolo clay loam after remaining in a moist chamber for different periods of time, after being centrifuged, indicates that the readjustment of moisture after the complete removal of the centrifugal force is slow. The distribution of moisture determined by slicing the samples with the microtome is shown in table 19.

TABLE 19

DISTRIBUTION OF MOISTURE WITHIN 60-GM. SAMPLES OF YOLO CLAY LOAM, AFTER BEING CENTRIFUGED AND ALLOWED TO REMAIN IN A MOIST CHAMBER FOR DIFFERENT PERIODS OF TIME

Results expressed in percentage of moisture on basis of oven-dry soil.

(Layer No. 1 is nearest the axis of rotation, No. 8 farthest from it.)

Each layer is 2 mm. thick.

Number of layer.....	1	2	3	4	5	6	7	8
Samples sliced between 11:04 a.m.* and 12:02 p.m.	20.22 ±0.046	20.36 ±0.035	20.87 ±0.098	21.12 ±0.041	21.32 ±0.064	21.23 ±0.086	21.15 ±0.107	22.76 ±0.096
Samples sliced between 12:15 p.m. and 1:40 p.m.	19.71 ±0.081	20.42 ±0.052	20.74 ±0.092	21.05 ±0.103	21.21 ±0.041	21.26 ±0.119	21.16 ±0.083	22.57 ±0.101
Samples sliced between 1:50 p.m. and 2:19 p.m.	20.46 ±0.036	20.70 ±0.033	20.57 ±0.254	21.22 ±0.113	21.43 ±0.072	21.42 ±0.058	21.40 ±0.044	22.81 ±0.077

\*All samples were removed from centrifuge and placed in moist chamber at 11:00 a.m.

There is no significant difference between the distribution of moisture in samples sliced within the first hour after removal from the centrifuge and that within samples sliced two or three hours later. The centrifuge was stopped at 11:00 a.m. The moisture distribution was the same in the samples sliced at 11:04 a.m. as that in those sliced at 2:19 p.m. The data presented in table 7 also indicate that no readjustment has taken place between the different layers within the time the sample in cup number 34 was sliced, which was as soon as possible after being centrifuged, and sample in cup number 12 was sliced, the latter having been in a moist chamber for more than an hour after centrifuging. However, it is possible that a readjustment of the moisture in the innermost layers may take place immediately after the centrifuge is stopped and before the samples could be sliced.

Joseph and Martin<sup>9</sup> explain the decrease in moisture equivalent with increase in amount of soil in the following language: "It will be

seen that in each case the moisture equivalent diminishes with increasing quantity (thickness of layer) of soil taken, and this is doubtless due to the variation in the density of packing, which will increase in the bottom layers with increasing quantity of soil and consequently reduce the space available for water."

It is not clear whether they thought the outer, or their "bottom" layers, were so compact that the total pore space was the limiting factor. This would necessitate the belief that the outer layers were saturated. They report no data to show the relative amounts of water in the inner and outer portions of the samples. If by "the space available for water," they meant the "larger capillary spaces" described by Briggs and McLane<sup>3</sup> and not the total pore space,  $S$ , it follows that they believed  $P_w$  was less in the outer layers than in the inner layers.

The data presented in Section IV indicates that increasing the pressure on the soil decreases the amount of water retained. Unfortunately, no determinations were made of  $A_s$  for the weighted samples. It is therefore impossible to arrive at a definite conclusion as to the ratio of  $P_v$  to  $S$ . The value of  $\frac{P_v}{S}$  calculated by direct-micrometer-volume measurements for the 10-gm. sample of Yolo clay loam reported in table 18, the original data for which was taken from table 12, is 80.1 per cent. It must be remembered that this is the average value for the entire sample. This high percentage suggests that the outer layers of this sample may be saturated, or nearly so. It may be that the 10-gm. samples with superimposed weights, which doubtless were more compact, reported in table 16, were saturated and that the pore space limited the amount of water retained, or

$$P_v = S.$$

A decrease in  $P_v$  may follow compacting some soils without resulting in a saturated condition, by a reduction in size of the "larger capillary spaces" described by Briggs and Shantz<sup>3</sup> and mentioned heretofore. The term "capillary spaces" is used in the same sense that Briggs<sup>2</sup> defines it, namely, as follows: "By a capillary space is meant not any interstitial space in the soil structure, but only that portion of it which is near the points of contact of two soil grains."

VARIATIONS IN WEIGHTS OF SOIL IN SAMPLES AS MEASURED  
BY VOLUME

Since the percentage of moisture retained is dependent upon the amount of soil placed in the centrifuge cups, it is important to consider the method used to select the amount of soil to be placed in the cups. The weights of samples of the five soils for which data are reported in this paper have been obtained by the usual method of filling a measuring cup and striking off the soil level with the top of the cup. In addition, the weights of an equal volume of air-dry peat have been obtained. Amounts of Yolo clay obtained by measuring into the centrifuge cup gave a variation in weight from 28.41 gm. to 30.7 gm.; the Yolo silt loam 29.5 gm. to 30.7 gm.; the Yolo clay loam 29.4 gm. to 32.0 gm.; Twin Falls silt loam 29.6 gm. to 32.7 gm.; Oakley sand 36.7 gm. to 41.8 gm.; and the peat 9.7 gm. to 10.3 gm. These soils were thoroughly pulverized and screened a number of times, whereas, in usual laboratory practice, this is not done so thoroughly. The results obtained in these trials may therefore be somewhat more consistent than in ordinary laboratory practice. For instance, the weights of dry soils in the samples used for sixteen determinations made on the same soil as given by Briggs and McLane<sup>4</sup> vary from 27.95 gm. to 34.1 gm.

The difference observed in selecting samples of Yolo clay by the use of the measuring cup, which resulted in the variation of weights of 2.3 gms according to our experiments, would result in a difference of 0.5 per cent in moisture equivalent. The observed difference for Yolo silt loam of 1.2 gm. in the volume measured samples would result in a difference in moisture equivalent of about 0.3 per cent. The difference of 2.6 gm. for the Yolo clay loam would give a difference of 0.8 per cent in moisture equivalent; Twin Falls silt loam, with an observed difference of 3.1 gm. will give a difference of 0.9 per cent. The observed difference of 5.2 gm. for the Oakley fine sand is equivalent to a difference in moisture equivalent of 0.3 per cent. These values are taken from the curves of figure 1.

The differences, as indicated in weights of samples of different soils obtained by measuring the samples by volume with a measuring cup, present a difficulty in making moisture equivalent determinations on a comparable basis between soils of extreme types. In this



connection, the tendency for the curves representing the finer soils used in these trials to converge, as shown in figure 1, when greater weights of soils are used, suggests the reduction of differences due to soil type in the moisture retained against the "centrifugal" force.

## SUMMARY

1. The moisture equivalent is materially influenced by the amount of soil used in its determination.

2. The smaller the sample the larger the percentage of water retained. The difference is greater with small samples than with large.

3. Some clay soils become impermeable to water during centrifuging when the amount of soil placed in the centrifuge cup is increased.

4. Some clay soils will reabsorb the supernatant water when the centrifugal force is relieved.

5. Within a 60-gm. block of soil, the moisture content increases from the inner surface to the outer, but the rate of increase is lower than that resulting from reducing the size of sample from 60 gm. to 5 gm.

6. Within blocks of soil much larger than 60 gm., the amount of water retained probably increases from the inner surface to the outer surface, if the soil is centrifuged long enough to establish equilibrium.

7. The apparent specific gravity of a 60-gm. block of soil is significantly greater than that of a 10-gm. block.

8. As the size of the sample is decreased from a 60-gm. to a 10-gm. sample, the apparent specific gravity decreases continuously.

9. Within a 60-gm. sample, the apparent specific gravity increases significantly from the inner surface outward to a point about 6 mm. within the sample, beyond which it remains substantially constant to the outer surface.

10. With 5-, 10-, 20-, or 30-gm. samples, the placing of weights on the inner surface decreases the amount of water retained by the soil.

11. Subjecting the moist soil to centrifugal force until the moisture is in equilibrium with this force results in the establishment of an equipotential region throughout the block of soil.

12. The moisture equivalent centrifuge, together with the special microtome and methods herein described, suggest means for further study of the relation between the capillary potential and the moisture content of the soil.

13. In general, the average pore space does not limit the amount of water retained by the soil block.

14. It is probable that the outer layer of the fine textured soils is saturated, or nearly so.

15. When precision is desired, it is necessary to measure the sample by weight rather than by the measuring cup.

16. The variation in moisture equivalent with samples of different sizes is influenced by the degree of compactness as well as by the depth of the sample in the centrifuge cup.

17. It is apparent from the data presented in this paper that further standardization of the moisture equivalent method is necessary if comparable results are to be obtained.

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## PLATE 1

Fig. 1. Special microtome with which the soil blocks were sliced into thin layers after centrifuging. One centrifuge cup from which the soil block has just been taken and another containing soil as it has come from the centrifuge are shown at the left.

Fig. 2. To assure uniformity in thickness of layer a razor having a rigid edge was used to slice the blocks.

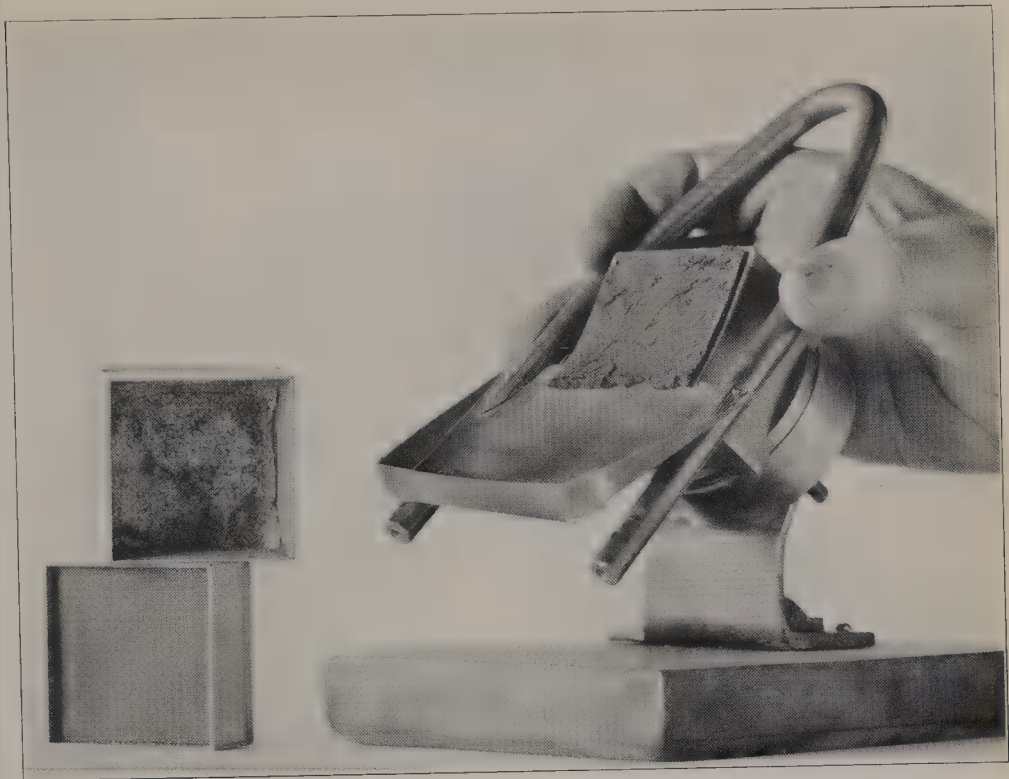


Fig. 1

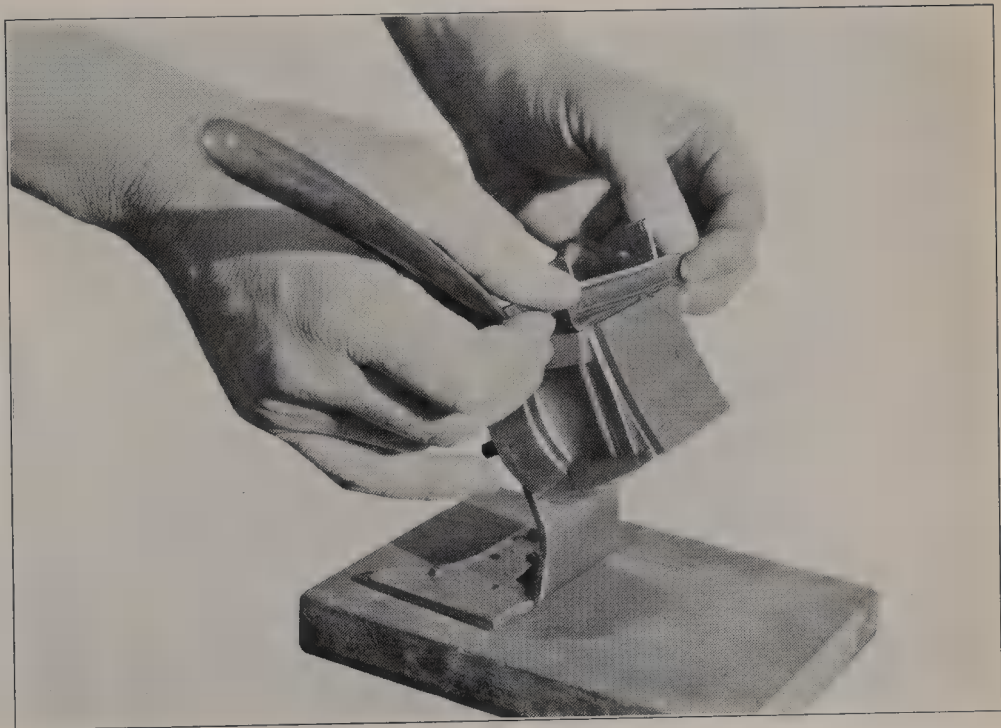


Fig. 2



## PLATE 2

Fig. 1. Method of storing samples in a moistened condition previous to centrifuging. These cups constitute one centrifuge set. In this instance four cups each of 30, 40, 50, and 60 gm. of Yolo clay loam are shown.

Fig. 2. These special guides made it possible to press the back of the razor on their curved surfaces and thus avoid variation in the thickness of slice due to variation in the pitch of the razor blade.



Fig. 1

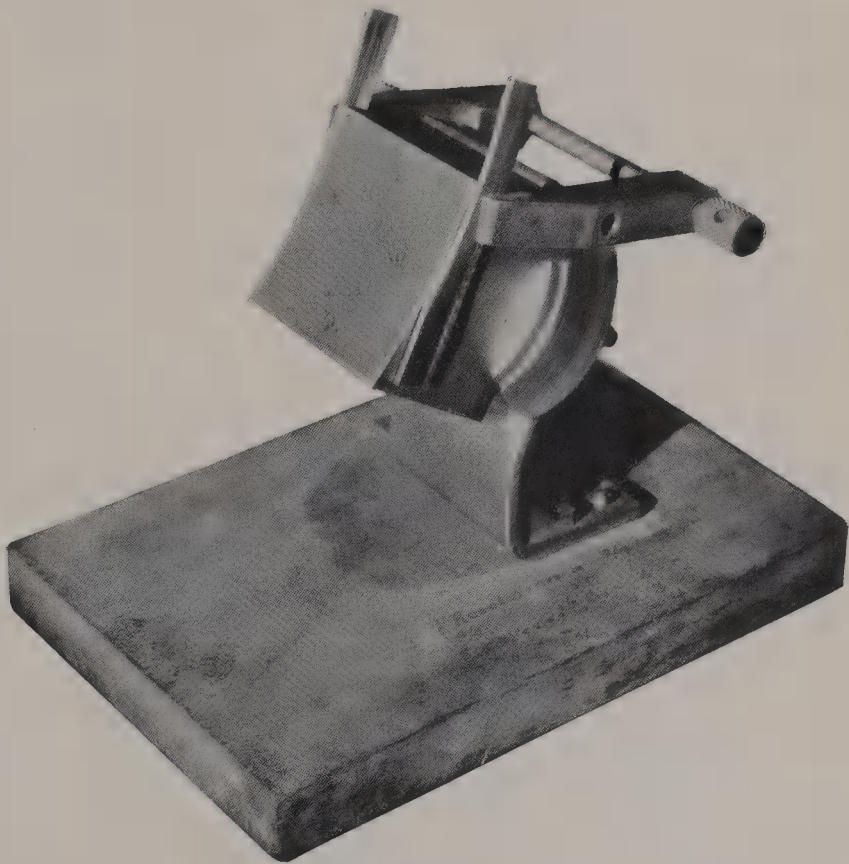


Fig. 2



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NUTRIENT AND TOXIC EFFECTS OF CERTAIN IONS  
ON CITRUS AND WALNUT TREES WITH ESPECIAL  
REFERENCE TO THE CONCENTRATION AND  
 $P_H$  OF THE MEDIUM

BY

H. S. REED AND A. R. C. HAAS

UNIVERSITY OF CALIFORNIA PRINTING OFFICE  
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---

INTRODUCTION

The continuation of studies which have been presented in publications from this laboratory<sup>17, 18, 19</sup> has led to the results presented in this paper which should be regarded as a report of work in progress.

The absorption of ions and their distribution in the plant are not determined by such relatively simple factors as the concentration or the solubility of the substance in question, but are profoundly influenced by the coördinating activities of the living plant. An ion may have a specific effect upon a single organ and have little or no direct effect upon others. It is therefore important to study the activity of the plant in conjunction with its chemical analysis, if we are to appreciate the equilibrium existing between the plant and its surroundings. The farmer is obviously interested in maintaining an equilibrium suited to the proper physiological functioning of the plant. The nutrient or toxic effects of certain of the compounds to be discussed are problems of agriculture in semi-arid regions where irrigation is practiced. The present studies are intended to extend our knowledge of the effect of certain factors upon the growth and composition of

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\* Paper No. 110, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

young citrus and walnut trees. The present conditions of experimentation in this field of investigation are such that it is necessary to employ young seedling plants for some of the work. It is hoped that the investigation may be extended to include experiments on the absorption process in older trees.

## EXPERIMENTAL DATA AND DISCUSSION

### I. THE EFFECT OF SODIUM SULFATE ON WALNUT SEEDLINGS

#### *(Juglans regia)*

The following experiments were designed to show the effects of sodium sulfate added to a well-balanced nutrient solution (Hoagland's). The seedlings were grown two months in 40-liter water cultures in the glasshouse. Twelve uniform walnut seedlings whose radicles were from 6 to 10 cm. long were suspended in each culture through holes in paraffined wooden covers which supported the young plants. Two jars were used for each concentration of the culture solution. The solutions were changed at the end of the first month, more frequent changes being considered unnecessary since the concentrations were high and the volume of the solution per plant large. The volume of the solutions was maintained by frequent additions of distilled water. The illustrations show that walnut seedlings may be successfully grown in a nutrient solution by this means.

The seedlings of series A (table 1) grew in Hoagland's nutrient solution, which has the following composition expressed as parts per million:

Na	K	Ca	Mg	Fe	Mn	NO <sub>3</sub>	Cl	SO <sub>4</sub>	PO <sub>4</sub>	Total
7	185	159	54	1	0.1	718	10	216	105	1455.1

Series B, C, D, and E contained this solution plus 1500, 3000, 4500, and 6000 p.p.m. sodium sulfate, respectively. Since the highest total concentration was approximately 7500 p.p.m., we employed another series (series F) which contained the same salts as series A, but in five-fold concentration.

The seedlings made very satisfactory growth in series A, B, and F (fig. 1). In the higher concentrations the growth of tops and roots was restricted, and the leaf margins were dead like those shown in

TABLE 1

GROWTH AND COMPOSITION OF WALNUT SEEDLINGS IN NUTRIENT SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF SODIUM SULFATE

Series	A		B		C		D		E		F	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Parts per million Na in culture solution.....		96		100		80		63		2007		35
Parts per million SO <sub>4</sub> in culture solution.....										4388		1080
Parts per million total solutes in culture solutions.....										7627		7275
Fresh weight per 10 plants (grams).....	114	6.73	97	6.71	55	8.42	26	8.34	22	7.13	93	110
Constituents of the dry matter (per cent of ash).....		3.43		4.85		5.92		4.87		4.74		10.44
Non-volatile SO <sub>4</sub> .....	10	.11	.35	.58	.75	.95	.90	.82	.78	.81	.56	.48
Total sulfur (expressed as SO <sub>4</sub> ).....	45	.47	.68	.79	1.07	1.14	1.29	1.33	1.17	1.54	.79	.93
Na.....	6.95	8.47	10.29	24.31	11.93	26.02	19.97	26.11	22.68	25.25	8.98	5.13
K.....	29.37	17.69	32.47	12.53	26.81	11.22	24.21	11.34	25.35	10.84	29.92	19.07
Ca.....	6.78	4.65	5.69	2.33	3.31	.67	2.24	1.56	1.54	1.38	6.94	3.93
Mg.....	3.60	1.75	3.80	1.17	2.85	.81	2.86	1.46	2.39	1.31	3.79	1.27
Cl.....	1.12	.93	1.23	2.09	.94	1.88	1.13	1.54	1.08	1.45	.94	1.01
SO <sub>4</sub> .....	1.96	3.24	5.16	12.04	8.83	16.07	10.78	16.73	10.96	17.14	5.29	4.63
PO <sub>4</sub> .....	16.24	51.77	17.88	39.21	17.61	34.61	20.64	41.00	17.71	41.65	17.39	32.51

fig. 2. The roots were less profusely branched and were dark colored. Growth of the lateral rootlets soon stopped and their apical portions were swollen like those shown in fig. 3. The seedlings of series F showed no abnormality although the initial concentration was 7275 p.p.m.; they differed from those grown in series A chiefly in the smaller growth of epicotyls and leaves.

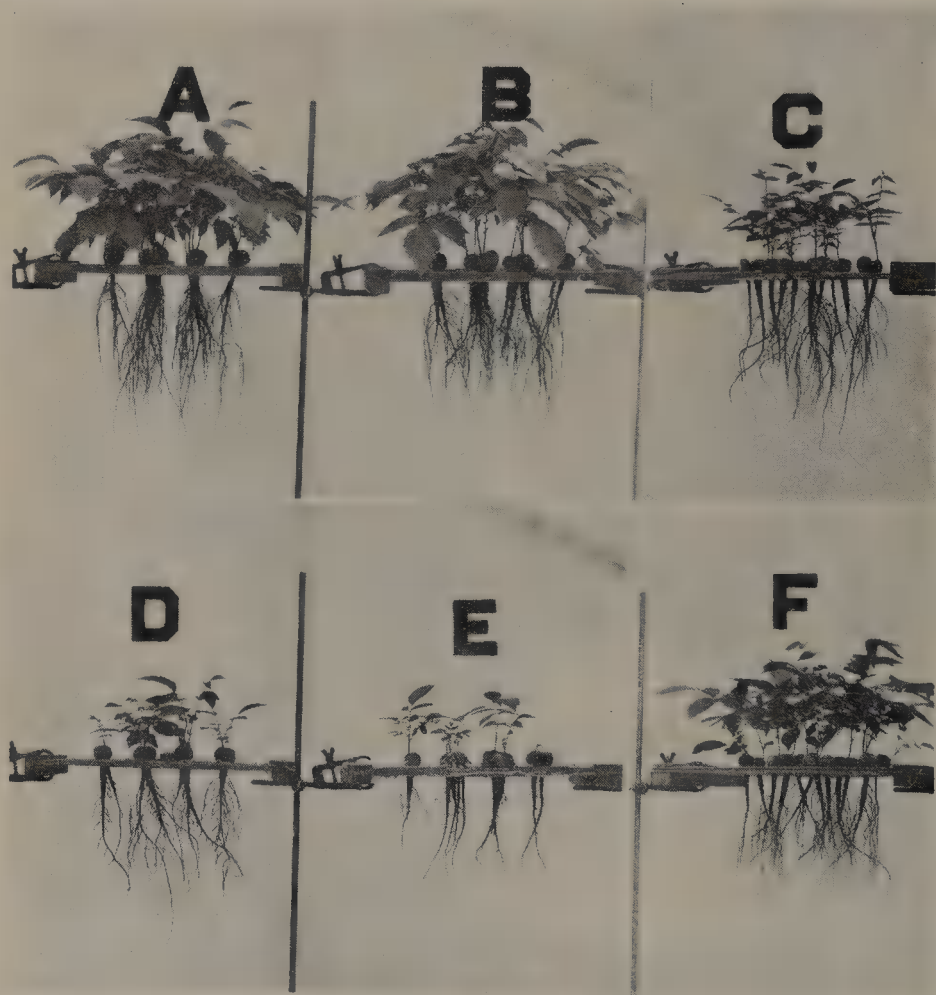


Fig. 1. Walnut seedlings which had grown two months in nutrient solutions containing sodium sulfate. A, in single-strength nutrient solution; B, C, D, E, in single-strength nutrient solution plus 1500, 3000, 4500, and 6000 p.p.m. sodium sulfate respectively; F, in nutrient solution having five times the ordinary concentration.

The fresh weight of the tops and roots of the plants (without cotyledons) in each series is given in table 1. It will be noted that growth of tops was more retarded by the higher concentrations of  $\text{Na}_2\text{SO}_4$  than that of roots, in fact the epicotyls entirely failed to

develop in some plants in the higher concentrations. The maximum ash content was found in the plants grown in series F.

The sulfur content of the plants, whether measured by the non-volatile  $\text{SO}_4$  or as total sulfur in the dry matter, reflected in a general way the amount of sulfate in the culture solution. There appears to



Fig. 2. Walnut leaf from plant grown in culture solution containing 4500 p.p.m. sodium sulfate. The dead margins were characteristic of the plants grown in the higher concentrations of sodium sulfate.



Fig. 3. Walnut root from culture solution containing more than 4000 p.p.m. sodium sulfate.

have been an increase in both the inorganic and organic compounds of sulfur.

The analyses of the ash show that active absorption of certain ions took place. The percentages of Na and of  $\text{SO}_4$  reflected the influence of these ions in the culture solutions up to a certain limit, beyond which an increase in the concentration had no effect.



The ash of the roots contained a higher per cent of Na than the tops with the exception of plants in series F. Where the culture solution contained 500 or more p.p.m. Na the per cent of this ion found in the ash of roots was nearly constant.

The ash of the tops contained a higher percentage of K than that of the roots. Although the percentage of K in the tops was fairly uniform in all series, the percentage of K in the roots was lower when the concentrations of  $\text{Na}_2\text{SO}_4$  in the solutions were higher.

The percentages of Ca and Mg although small, show a rather striking relation to the amounts of  $\text{Na}_2\text{SO}_4$  in the culture solution. The higher concentrations of  $\text{Na}_2\text{SO}_4$  seem to have diminished the absorption of Ca and Mg.

The roots were richer in  $\text{SO}_4$  than the tops, except in series F. Series C, D, and E were rather uniform in size (cf. fig. 1) and contained considerably higher amounts of  $\text{SO}_4$  (series A, B, and F). With the data at hand it is not possible to relate the growth entirely to the amounts of Na or of  $\text{SO}_4$  found in the plants. We are inclined to believe that the retarded growth in series C, D, and E is correlated to some extent with the lower percentages of Ca absorbed by the plants. In view of the conspicuous changes in the size and shape of lateral roots of the injured plants it is not improbable that their ability to absorb ions was modified because of altered arrangement and structure of the cells. The microscopical structure of the malformed roots awaits study.

## II. THE EFFECT OF SODIUM NITRATE ON WALNUT SEEDLINGS

Walnut seedlings were grown from April 11 to June 5 as water cultures in 2-quart glass jars, with one seedling in each jar. Each series contained 12 jars. The initial concentration of Na and  $\text{NO}_3$  is given in table 2. The solutions were renewed every two or three weeks.

With solution G (table 2) as a basis the other solutions were made by adding 1324, 2647, 3971, and 5295 parts per million of  $\text{NaNO}_3$ , respectively. The total concentrations are given near the top of table 2.

The growth of the walnut seedlings was similar to that in the preceding experiment, although the roots were less affected by the higher concentrations of  $\text{NaNO}_3$ . The greatest effect was noted in

TABLE 2

GROWTH AND COMPOSITION OF WALNUT SEEDLINGS IN NUTRIENT SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF SODIUM NITRATE

Series	G		H		I		J		K	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Parts per million Na in culture solution.....		7		366		724		1083		1442
Parts per million NO <sub>3</sub> in culture solution.....		718		1683		2648		3613		4578
Parts per million total solutes in culture solution..		1455		2780		4102		5426		6750
Fresh weight per 10 plants (grams).....	77	92	72	90	46	75	36	71	31	90
Constituents of the dry matter (per cent):										
Ash.....	4.88	3.46	5.25	3.95	5.64	4.16	5.98	4.82	7.32	5.28
Total N.....	2.68	3.31	3.16	3.63	3.54	4.17	3.80	4.66	3.76	4.73
Constituents of the ash (per cent):										
Na.....	6.49	7.92	12.88	15.76	16.53	16.47	18.78	22.65	21.01	21.43
K.....	26.78	25.86	27.68	19.05	26.80	15.75	21.07	18.07	19.18	17.53
Ca.....	8.09	2.92	6.17	1.45	3.21	0.99	3.32	0.78	2.39	1.18
Mg.....	4.20	2.38	3.67	1.08	2.56	1.18	2.60	1.18	1.84	1.56
SO <sub>4</sub> .....	2.57	3.74	2.98	5.26	2.88	7.08	3.90	8.75	3.10	8.18
PO <sub>4</sub> .....	19.92	54.95	18.78	48.90	19.66	50.68	21.40	46.17	18.27	42.71

the tops of the seedlings. The leaves of plants in series I, J, and K showed dead margins somewhat as illustrated in fig. 2. The injury was increasingly severe in the higher concentrations of  $\text{NaNO}_3$ . Since the seedlings in solution F (table 1) showed no dead leaf margins when the total concentration of salts was 7275 p.p.m., it is improbable that the injury was caused solely by concentration. The root systems were well developed and healthy in appearance. The size of the plants is indicated by the fresh weights. It is interesting to note that the growth in series H was as good as in G, although the former solution contained over 1600 p.p.m.  $\text{NO}_3$ . The inhibiting effects of larger concentrations of  $\text{NaNO}_3$  appear to be shown mainly by the epicotyls.

The percentages of ash and of nitrogen were increased by the higher concentrations of  $\text{NaNO}_3$  although not proportionally to the concentration.

The percentage of Na in the tops and roots increased up to a certain point, beyond which no significant increase was found. The percentages of Ca and of Mg were substantially reduced by the more concentrated solutions.

In another experiment, walnut seedlings were grown two months in Hoagland's nutrient solution which contained  $\text{NaNO}_3$  or  $\text{Na}_2\text{SO}_4$  in concentrations of  $\frac{M}{46}$ . The solutions were renewed every two or three weeks. Duplicate cultures were made in stone jars holding 40 liters with 13 seedlings in each jar.

Both series made fairly good growth, though the leaves in the  $\text{Na}_2\text{SO}_4$  series showed some dead margins. The roots in both sets developed normally. The general appearance of the seedlings at the end of two months is shown in fig. 4. The fresh weight of 10 plants from the  $\text{NaNO}_3$  series was 194 g. and that of 10 plants from the  $\text{Na}_2\text{SO}_4$  series was 132 g.

The interpretation of this experiment is complicated by the fact that to one solution twice as much Na was added as to the other, but with these equi-molecular solutions the growth was better in the  $\text{NaNO}_3$  set.

The influence of the cation on walnut seedlings was further studied by an experiment consisting of three series of cultures. The first contained Hoagland's nutrient solution in four times the usual concentration; the second, Hoagland's nutrient solution (ordinary strength) plus 2692 p.p.m.  $\text{NO}_3$  as  $\text{Ca}(\text{NO}_3)_2$ ; the third, Hoagland's nutrient

solution (ordinary strength) plus 2692 p.p.m.  $\text{NO}_3$  as  $\text{NaNO}_3$ . The seedlings were grown for six weeks and the fresh weights of 10 plants were 150 g., 152 g., and 84 g., respectively.

These results indicate that, in these high concentrations Na was much less favorable to growth than Ca, and that  $\text{SO}_4$  is more toxic than equivalent concentrations of  $\text{NO}_3$ .



Fig. 4. Walnut seedlings grown two months in nutrient solutions plus equi-molecular concentrations of sodium salts.

N  $\frac{\text{M}}{46}$  sodium nitrate; S,  $\frac{\text{M}}{46}$  sodium sulfate.

TABLE 3  
EFFECT OF DIFFERENT CONCENTRATIONS OF CULTURE SOLUTION CONTAINING 500 PARTS PER MILLION NaCl, UPON  
GROWTH AND COMPOSITION OF ORANGE SEEDLINGS

Series	L		M		N		O	
	Culture solution		Culture solution plus 500 p.p.m. NaCl		Double-strength culture solution plus 500 p.p.m. NaCl		Triple-strength culture solution plus 500 p.p.m. NaCl	
Total concentration of solutes (p.p.m.).....	1455		1954		3408		4862	
Fresh weights of 100 plants (grams) .....	Tops    Roots		Tops    Roots		Tops    Roots		Tops    Roots	
Dry weights of 100 plants (grams).....	227    160		169    113		236    136		192    108	
	72    33		50    25		66    29		57    25	
Per cent of ash in the dry matter.....	10.40		11.94		12.39		13.32	
Na (per cent of ash).....	5.22		13.13		11.57		9.78	
Cl (per cent of ash).....	.75		9.52		7.94		6.86	



TABLE 4  
COMPARATIVE EFFECTS OF NaCl AND CaCl<sub>2</sub> UPON GROWTH AND ABSORPTION BY WALNUT SEEDLINGS

Series	P		Q		R		S		T	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
P.p.m. Cl added to the culture solution.....	1542	1542	1542	1542	1542	1542	1542	1542		
P.p.m. Na added to the culture solution.....	1000	1000	0	0	1000	1000	0	0		
P.p.m. Ca added to the culture solution.....	0	0	871	871	0	0	871	871		
Concentration of nutrient solution (p.p.m.).....	1455	1455	1455	1455	7275	7275	7275	7275	5820	5820
Total concentration of solutes (p.p.m.).....	3997	3997	3868	3868	9817	9817	9688	9688	5820	5820
Fresh weights per 10 plants (grams) .....	27	57	37	61	16	38	19	40	82	68
Per cent of ash in the dry matter.....	7.66	4.80	9.51	5.50	9.62	6.54	9.27	7.32	9.66	8.10
Constituents of the ash (per cent):										
Na.....	15.16	18.94	5.70	7.21	13.73	9.14	5.76	3.28	8.19	6.48
K.....	26.68	17.05	25.20	22.88	30.83	20.05	26.30	17.93	30.83	19.59
Ca.....	4.66	1.20	12.64	4.96	3.60	2.70	7.39	5.77	6.51	9.04
Mg.....	3.09	1.42	2.34	2.13	2.59	3.10	2.55	2.52	3.61	1.75
Cl.....	10.17	9.23	12.87	10.10	9.30	6.01	9.29	3.86	1.24	1.60
SO <sub>4</sub> .....	3.41	2.91	2.33	3.48	5.31	4.13	5.13	5.40	4.34	6.82
PO <sub>4</sub> .....	23.13	47.52	15.45	39.82	21.01	50.34	20.81	49.00	2.65	5.10

### III. THE ABSORPTION OF SODIUM AND CHLORIN FROM NUTRIENT SOLUTIONS OF DIFFERENT CONCENTRATION

It has been known for a long time that within a certain range there is some sort of relation between the concentration of ions in a solution and the amount absorbed. The data given above confirm the earlier results on this particular point. We wish to present some data which show that the absorption of an ion is affected by the concentration of other ions. In this instance 500 parts per million NaCl were added respectively to several concentrations of standard nutrient solution.

The experiments were made with St. Michael orange seedlings. Series L (table 3) contained Hoagland's nutrient solution during both periods of the experiment. Series M, N, and O contained Hoagland's solution plus 500 p.p.m. NaCl for 158 days (August 14 to January 19) and then the cultures were grown 33 days in solutions having increased concentrations of nutrient solution plus 500 p.p.m. NaCl. When the experiment was terminated no serious toxic effects were observed in any of the plants, although there were appreciable differences in the sizes of the plants.

Growth was most retarded in series M where the NaCl was added to single-strength nutrient solution. In series N the growth was practically as good as in series L which had no NaCl, which may indicate that the higher concentration of nutrient ions decreased the toxicity of the NaCl. In series O the plants were about the same as in series M, this fact possibly indicating that the concentration of solutes had in itself an inhibiting influence on growth. The percentage of ash in the dry matter progressively increased with increasing concentration of the solution. The percentages of Na and of Cl in the ash were greatest in series M with a progressive decrease in N and O. It would appear that there was something like a dilution of the NaCl due to the higher concentration of nutrient ions.

We may conclude therefore that (within certain limits of concentration) it is not alone the absolute amount of an ion that determines the amount that will be absorbed, but that its relation to the concentration of the other ions is also of considerable importance.

#### IV. THE CHLORIN CONTENT OF PLANTS GROWN IN SOLUTIONS OF DIFFERENT CHLORIDS

##### *a. Walnut seedlings.*

The question of the influence of cation upon the absorption of anion has been frequently raised in these and other experiments. Plate<sup>16</sup> observed that the growth of oat seedlings in gram-molecular solutions of chlorids of the different monovalent cations was by no means equal. The growth of tops in the various chlorids formed a descending series as follows:  $\text{NH}_4 > \text{K} > \text{Na} > \text{Rb} > \text{Li} > \text{Cs}$ . The growth of roots formed a similar series except that the positions of K and Na were reversed. Hoagland<sup>4</sup> found that there were no significant differences in the amounts of Cl absorbed from different salt solutions.

For the next experiment a concentration of 1542 p.p.m. Cl was used in conjunction with the standard nutrient solution (series P and Q, table 4) and with a five-fold concentration of the nutrient solution (series R and S). The control (series T) consisted of a four-fold concentration of nutrient solution. The figures at the top of table 4 show the initial amounts of the ions and the total concentration of the culture solutions. Four series of 26 walnut seedlings each were grown in 40-liter jars, from September 19 to October 30, the solutions being renewed every two to three weeks.

The seedlings in the first four series showed the toxic effects of the chlorids (fig. 5). The plants in series P (NaCl) were characterized by small leaves and a restricted growth of lateral roots, while those in cultures to which  $\text{CaCl}_2$  was added (series Q) produced good leaves and tops. The primary roots in the NaCl cultures were brown, while those in the  $\text{CaCl}_2$  cultures were white and thickened. The lateral roots in both series showed restricted growth (fig. 5), and those in series P (NaCl) were short and brown. The roots grew well in series Q ( $\text{CaCl}_2$ ) for a time, but decay set in at the upper end of the laterals near the primary root and eventually extended down to the tips. Growth in the more concentrated solutions (R and S) was more limited than in the other two series. The plants in the R series (NaCl) produced short epicotyls and small leaves. In the S series ( $\text{CaCl}_2$ ) the epicotyls were longer and the leaves were somewhat larger, but many leaves showed the type of "burning" which is characteristic of high salt concentrations.

Certain relations between absorption and composition of culture solution are shown in table 4. Attention is directed to the fairly constant ratio of chlorin to ash constituents in the four series. Where growth was approximately equal, there was practically the same absorption of Cl without regard to the amount of Na or Ca present. Plants in the concentrated solutions absorbed somewhat less Cl than those in the dilute solutions, probably as a result of the introduction of additional ions.



Fig. 5. Comparative effects of sodium chlorid and calcium chlorid on the growth of walnut seedlings. P, 1542 p.p.m. Cl as sodium chlorid plus single-strength nutrient solution; Q, 1542 p.p.m. Cl as calcium chlorid plus single-strength nutrient solution; R, 1542 p.p.m. Cl as sodium chlorid plus concentrated nutrient solution; S, 1542 p.p.m. Cl as calcium chlorid plus concentrated nutrient solution (cf. table 4).

The results of these series of cultures show that the toxic effects of NaCl and CaCl<sub>2</sub> on walnut seedlings, while similar, are not identical. In series P and Q, where the total concentration was not detrimental, NaCl appeared to check the growth of the tops more than CaCl<sub>2</sub>. In series R and S where the total concentrations were high, there was less difference in the effects of the two salts.

Another experiment with walnut seedlings was made to compare the effects of NaCl and KCl. Three series of cultures were made in 40-liter jars. The first received the nutrient solution in three times the ordinary concentration; the second and third received 1542 p.p.m.

Cl as KCl and NaCl respectively (table 5). The cultures were started December 27 and ended February 7 and the solutions were renewed every two or three weeks.

The growth of the seedlings in the NaCl series was somewhat better than that made in cultures conducted during the summer or early fall. The tops in the NaCl series showed no tendency towards burning like that formerly observed and the growth of roots was not so greatly retarded, although the development of lateral roots was conspicuously inferior to that of the control series (fig. 6).



Fig. 6. Comparative effects of sodium chlorid and of potassium chlorid on the growth of walnut seedlings. U, nutrient solution having three times the ordinary concentration; V, 1542 p.p.m. Cl as potassium chlorid plus nutrient solution; W, 1542 p.p.m. Cl as sodium chlorid plus nutrient solution.

TABLE 5  
GROWTH OF WALNUT SEEDLINGS IN NUTRIENT SOLUTIONS CONTAINING EQUIVALENT CONCENTRATIONS OF Cl FURNISHED AS NaCl OR AS KCl.

Series	U		V		W	
P.p.m. Cl added to the culture solution.....			1542		1542	
P.p.m. Na added to the culture solution.....					1000	
P.p.m. K added to the culture solution.....			1700			
Concentration of nutrient solution (p.p.m.).....	4365		1455		1455	
Total concentration of solutes (p.p.m.)....	4365		4697		3997	
	Tops	Roots	Tops	Roots	Tops	Roots
Fresh weight per 10 plants (grams).....	52	110	81	104	40	90



The leaves in the KCl series, although large and broad, were pale green and eventually became yellow. The roots, though well branched, stopped growing and began to die before the end of the experiment. The injury to the lateral roots, like that previously noted in toxic concentrations of  $\text{CaCl}_2$ , was first noticed in the region next to the primary root, from which it extended toward the apex. The plants in the control series were healthy during the entire period of the experiment and produced splendid root systems.

The quantitative relations of the three series are shown by the fresh weights of the plants given in table 5. The weight of roots showed no significant differences, but the weights of tops were quite different. The figures indicate a distinct toxic action of the NaCl and a pronounced increase in growth in the KCl series. The increase in the latter was made during the first part of the experimental period; subsequently, the toxic effects gradually appeared. The condition is somewhat similar to that seen when equivalent amounts of  $\text{CaCl}_2$  were employed in former experiments.

There is an obvious difference in this case between the toxic effects of NaCl and KCl when equivalent amounts of Cl are used. It might be concluded that an early toxic effect of Cl was more or less prevented by the K ions. It is obvious that there is a time factor involved in the case of toxicity. The toxic effect of  $\text{CaCl}_2$  appears later than that of NaCl, but it may eventually terminate growth of the plant.

#### *b. Citrus seedlings.*

A series of cultures of rough-lemon seedlings was grown for 32 days in a complete nutrient solution with the addition of sodium or calcium chlorid. Eighteen jars each containing three seedlings were used for each concentration. The controls were grown in nutrient solution; the second set was grown in nutrient solution plus 308, 617, 1234 or 1851 p.p.m. chlorin, as sodium chlorid; the third set of plants was grown in solutions the same as the second except that the chlorin was added as calcium chlorid.

The seedlings grown in solutions containing 308 p.p.m. sodium chlorid showed no injury, either to roots or tops; those in solutions containing 617 p.p.m. showed a small amount of injury to the older leaves. The symptoms of injury were a recurved condition and death of the tissues at the margin of the leaf. When the concentration was greater than 617 p.p.m. chlorin, the amount of injury was greater. There was an appreciable, although small, stimulation to growth in all

TABLE 6  
GROWTH AND COMPOSITION OF WALNUT SEEDLINGS IN RELATION TO EQUIVALENT AMOUNTS OF VARIOUS ANIONS FURNISHED WITH SODIUM

Series	AA		BB		CC		DD		EE	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
P.p.m. Na in culture solution.....	1000	2692 NO <sub>3</sub>	1000	1542 Cl	1000	2649 HCO <sub>3</sub>	1000	2086 SO <sub>4</sub>	28	
P.p.m. of anion added.....	1455	5147	1455	3997	1455	5104	1455	4541	5820	
Concentration of nutrient solution (p.p.m.).....									5820	
Total concentration of solutes (p.p.m.).....									5820	
Fresh weight per 10 plants (grams).....	44	40	41	63	20	54	42	52	82	68
Per cent of ash in the dry matter .....	8.26	6.24	8.28	5.96	6.59	4.88	7.66	6.30	9.66	8.10
Constituents of the ash (per cent):										
Na.....	17.25	23.10	17.38	21.38	25.34	24.54	15.24	25.41	8.19	6.48
K.....	24.41	12.34	26.90	11.86	28.18	11.39	29.28	7.73	30.83	19.59
Ca.....	5.57	2.83	3.34	1.87	1.46	2.61	3.83	1.72	6.51	9.04
Mg.....	3.40	1.24	2.32	.97	2.07	1.35	2.91	.91	3.61	1.75
Cl.....	1.04	2.29	13.22	14.48	.92	1.84	.98	1.86	1.24	1.60
SO <sub>4</sub> .....	2.74	9.18	3.06	3.91	4.32	9.73	9.52	16.89	4.34	6.82
PO <sub>4</sub> .....	8.83	11.55	9.30	12.71	21.16	11.15	5.73	8.15	2.65	5.10

of the jars which contained 2000 p.p.m. sodium chlorid. Equivalent concentrations of chlorin as calcium chlorid were somewhat less toxic to the plants. The nutrient solution employed contained 159 p.p.m. calcium, yet the addition of calcium chlorid appears to have had a distinctly beneficial effect upon the growth of the plants during the short period of the experiment. Valencia orange trees grown for a longer time in sand cultures receiving 1770 p.p.m. chlorin as calcium chlorid, have shown severe tip burn of the leaves with their subsequent abscission.

We have previously shown<sup>18</sup> that orange trees made poor growth in sand cultures when furnished 1000 p.p.m. NaCl, and that growth was much better when also 440 p.p.m. CaCl<sub>2</sub> were added. The difference should be referred to the fact that the first lot suffered, not only from the toxic action of NaCl, but also from the lack of Ca. The introduction of CaCl<sub>2</sub> improved conditions for growth in spite of the fact that it increased the concentration of Cl ions. So long as the concentration of Cl is not too high, the presence of Ca may tend to offset the injurious effect of Cl by promoting growth and hence diluting the concentration of Cl in the plant, but in higher concentrations of Cl, or with longer time, injury ultimately appears.

## V. THE EFFECT OF DIFFERENT SALTS OF SODIUM

We have discussed the effect of equal concentrations of Cl as NaCl and as CaCl<sub>2</sub>, and shall now consider the toxic effects of equal concentrations of Na when added in the form of NaNO<sub>3</sub>, NaCl, NaHCO<sub>3</sub>, or Na<sub>2</sub>SO<sub>4</sub>. Five series of walnut seedling cultures were prepared according to the plan shown at the top of table 6. Culture EE contained Hoagland's nutrient solution of four times the usual concentration and may be regarded as the control. Each series contained 26 walnut seedlings, which were grown from July 17 to September 1. It will be seen that each of the four toxic solutions contained 1000 p.p.m. Na. Previous experiments had shown that this concentration of Na salts is decidedly toxic to walnut seedlings. The control solution had only 28 p.p.m. of Na, but its total concentration of solutes was 5800 p.p.m. at the outset. In spite of this high concentration there was no evidence of harmful effect.

The general appearance of the plants from the different solutions at the end of the experiment is shown in fig. 7, and the fresh weights of tops and roots are shown by diagram 1.



Fig. 7. Walnut seedlings showing effects of 1000 p.p.m. sodium in combination with different anions. AA, sodium nitrate; BB, sodium chlorid; CC, sodium bicarbonate; DD, sodium sulfate; EE, nutrient solution having four times the ordinary concentration (cf. table 6).

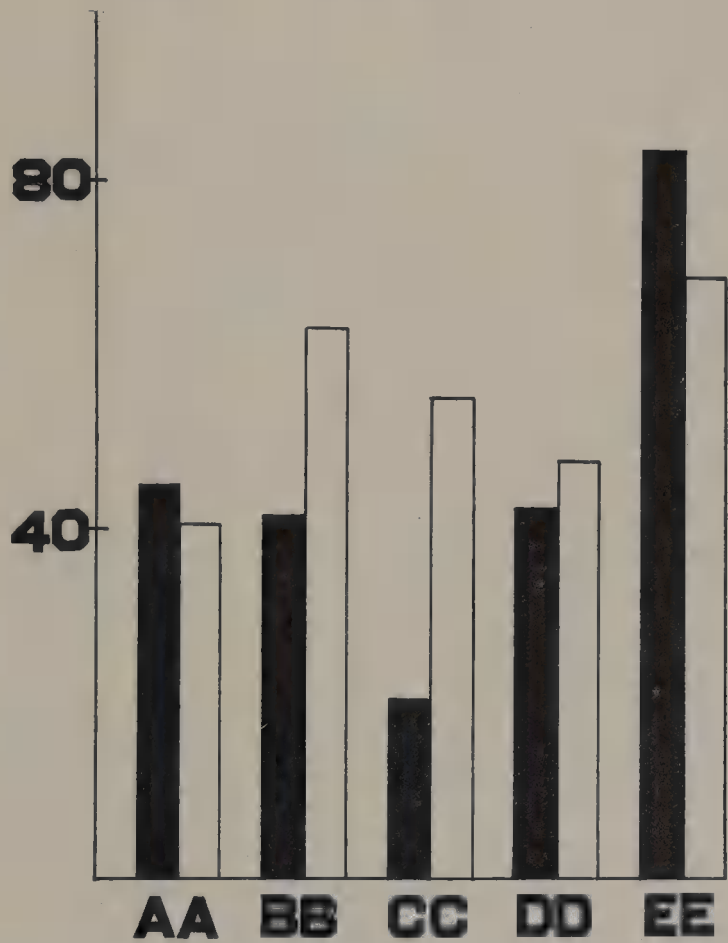


Diagram 1. Representation of the comparative growth of walnut seedlings from cultures in which different sodium salts were present in equivalent amounts. Shaded columns represent fresh weight of tops; unshaded columns, fresh weight of roots. AA, sodium nitrate; BB, sodium chlorid; CC, sodium bicarbonate; DD, sodium sulfate; EE, controls. Vertical scale represents green weight in grams.

The growth of the epicotyls may indicate the comparative toxicity of the various solutions, since practically no epicotyls had developed when the seedlings were placed in the cultures. Their development was most retarded in the  $\text{NaHCO}_3$  series. In the  $\text{NaNO}_3$  and the  $\text{NaCl}$  series, the margins of some of the leaves died showing what may be called salt burn. In the  $\text{NaHCO}_3$  series the leaves on the epicotyls were small, and in many cases the epicotyls did not emerge from the seed.

The primary roots (initially 8 to 15 cm. long) made small growth in all the Na series. The laterals produced later were short and swollen at the tips like those shown in other experiments with toxic salts. When the experiment was ended the roots in the toxic solutions were brown, though still living. The plants in the control series had well developed leaves and healthy white roots. Some idea of the toxicity of 1000 p.p.m. Na may be obtained by comparing the growth of seedlings in the  $\text{NaNO}_3$  series with that in the control series. In the former there was a  $\text{NO}_3$  concentration of 2692 p.p.m., while in the latter the  $\text{NO}_3$  concentration was 2872 p.p.m. In another experiment a concentration of 2692 p.p.m.  $\text{NO}_3$  added to the same nutrient solution in the form of  $\text{Ca}(\text{NO}_3)_2$  gave as good growth as the control solution.

The analyses recorded in table 6 show that the Na content of the tops and roots was high and rather uniform when the seedlings had grown in the  $\text{NaNO}_3$ ,  $\text{NaCl}$ , or  $\text{Na}_2\text{SO}_4$  series. Those grown in  $\text{NaHCO}_3$  had more Na in their tops than the other plants, but had practically the same percentage in the roots. It will be noted that the tops which made the least growth had the most Na in their ash. The ash of plants from the control series contained approximately the same percentage of Na as one usually finds in similar plants which have grown in less concentrated nutrient solutions.

The K content of the tops was reasonably uniform, though there was some reduction in the K content of the roots.

The effects of  $\text{NaNO}_3$ ,  $\text{NaCl}$ , and  $\text{Na}_2\text{SO}_4$  were not widely different, but  $\text{NaHCO}_3$  was more toxic than the other three salts used. Both the tops and roots of the seedlings showed qualitative differences which were not always evident from the weights of those parts. We have found that seasonal conditions may influence the results.

An earlier experiment in which nontoxic concentrations of salts were employed may be cited.<sup>20</sup> Rough-lemon seedlings were grown in nutrient solution containing equivalent concentrations of calcium



in conjunction with  $\text{NO}_3$ ,  $\text{SO}_4$ ,  $\text{Cl}$ , and  $\text{CO}_3$ . In the concentrations employed, the growth of roots was influenced more by the amount of calcium in solution than by the character of the anion with which the calcium was combined.

The effects of anions upon the growth of oat seedlings reported by Plate<sup>15</sup> afford an interesting comparison, although he used dilute acids rather than salts, and root growth was inhibited in all the solutions. The size of the oat shoot at the end of the fourteenth day in the different solutions was as follows:  $\text{PO}_4 > \text{SO}_4 > \text{NO}_3 > \text{Cl}$ .

## VI. THE EFFECT OF ALKALINITY ON GROWTH AND SAP REACTION

### *a. Walnut seedlings.*

The object of this experiment was to test the effect of a  $\text{Ca}(\text{OH})_2$  solution maintained at  $\text{P}_\text{H}$  9.0 upon the reaction of the sap of roots and tops. Two jars of 40-liter capacity were filled with tap water to which  $\text{Ca}(\text{OH})_2$  solution was added. Twelve walnut seedlings were placed in each jar through perforated covers so that their roots were immersed in the solution. They grew from April 13 to May 9. The  $\text{P}_\text{H}$  of the solution was maintained at or very near 9.0 by allowing a slow stream of saturated  $\text{Ca}(\text{OH})_2$  solution to flow continually into the culture solution.<sup>21</sup> The  $\text{P}_\text{H}$  of the culture solution was tested at least three times a day and the flow of the  $\text{Ca}(\text{OH})_2$  solution was regulated accordingly.

During the 26 days of this experiment the plants showed no injury, the roots were well developed though the tops seemed spindling. The fresh weights of tops and roots of 10 seedlings were 53 and 92 grams, respectively. The  $\text{P}_\text{H}$  of the juice expressed from the tops and roots was determined by means of the hydrogen electrode, and was 5.26 and 5.48, respectively. The acid nature of the plant juices agrees with our former determinations and with those of Theron.<sup>25</sup> It seems that the plant contains substances capable of acting as buffers which prevent the change of reaction in the sap.

Although the per cents of Ca found in ash of the tops and roots were 14.64 and 9.80, respectively, nevertheless the  $\text{P}_\text{H}$  of their sap showed no significant changes from the values obtained when such plants are grown in complete culture solutions.

In another experiment walnut seedlings were grown in tap water containing enough  $\text{Ca}(\text{OH})_2$  to raise the  $\text{P}_\text{H}$  considerably above 9.0. Within a few hours the roots were badly discolored; shortly afterwards

the laterals and the apical portion of the main root died. No new additions of  $\text{Ca}(\text{OH})_2$  solution were made and four days later the  $P_H$  of the solution had fallen to 7.2.

After the extreme alkaline conditions disappeared new laterals were produced not only from the white portions of the primary roots of the seedlings but also from the discolored parts of the primary roots, indicating that in the injured portion at least parts of the vascular system were still alive. The subsequent growth of laterals was very good. The original injury appears to have been confined to the cells of the parenchyma and meristem. Supplementary evidence on this question was afforded by the recovery of roots which had been held for a short time in culture solutions having a slightly injurious reaction. The surface of the roots was discolored. In a short time the reaction was brought to a  $P_H$  near 9.0 at which the roots resumed growth.

The subsequent elongation of the central column of root cells broke the discolored layer of cortical cells in the region of greatest growth and produced a banded surface. Similar injury and recovery has been observed when roots had been kept in solutions containing chlorids or other deleterious salts.

#### *b. Wheat seedlings.*

Four 40-liter jars were planted with small wheat seedlings. Two culture jars contained a modified nutrient solution maintained as near  $P_H$  6 as possible, the other two jars contained the same modified solution to which enough  $\text{Ca}(\text{OH})_2$  solution was added to maintain a  $P_H$  of 8.0. The composition of the modified nutrient solution expressed as p.p.m. was as follows:

Na	K	Ca	Mg	Fe	Cl	$\text{NO}_3$	$\text{SO}_4$	$\text{PO}_4$	Total
7	496	33	54	1	10	819	216	105	1741

The  $P_H$  of the solutions was adjusted three times each day and the solutions were renewed every two or three weeks during the two months of the experiment. Sufficient ferric tartrate was added periodically to prevent the appearance of chlorosis.

After two months the green weight of 20 plants from the culture at  $P_H$  6.0 was 209 g.; the same number from the culture at  $P_H$  8.0 weighed 498 g. It is quite likely that part of the increased growth at the higher  $P_H$  was due to increased supply of Ca. The plants from both cultures appeared healthy.

The data in table 7 show that there was no effect on the  $P_H$  of tops due to the addition of  $Ca(OH)_2$  to the culture solution, but that the  $P_H$  of roots was slightly affected. Hurd<sup>7</sup> and Haas<sup>2</sup> have reported an increased  $P_H$  in the tops of wheat plants grown in soil to which calcium carbonate was added. On the other hand Newton<sup>14</sup> and the writers found no such increase when plants were grown in water cultures. It would appear that the plants contain substances acting as buffers which maintain a constancy of reaction in spite of differences in the reaction of the medium or of changes in the ion composition of the sap. Similar conclusions were given in another paragraph based upon studies of the sap of walnut seedlings grown at different  $P_H$ .

TABLE 7  
THE EFFECT OF ADDED  $Ca(OH)_2$  UPON WHEAT SEEDLINGS

$P_H$ of culture solution.....	6.0	8.0
Green weight of tops per 20 plants.....	173 g.	440 g.
Green weight of roots per 20 plants.....	36 g.	58 g.
$P_H$ of sap of tops.....	6.1	6.1
$P_H$ of sap of roots.....	6.7	7.2
Calcium content of 20 c.c. sap from tops.....	.0017 g.	.0027 g.

## VII. THE RELATION OF H-ION CONCENTRATION TO THE ABSORPTION OF CHLORIDS

### a. *Citrus seedlings.*

The experiments were designed to determine the amount of chlorin absorbed by citrus seedlings from solutions of various H-ion concentration. Rough-lemon seedlings (*Citrus limonia* Osbeck) were grown and all of the plants were from seeds of a single tree. The cultures were grown in enameled-ware pails of ten liters capacity, each of which had approximately 65 seedlings supported on a perforated wooden cover (fig. 8). The solutions were renewed at intervals of three to four days and their reactions were adjusted three times each day.\*

The culture solutions was based on that employed by Hoagland. Sodium chlorid was added in amounts equivalent to 1000 p.p.m. (table

\* The writers are indebted to A. E. Michelbacher for the careful manipulation of details connected with this work.

8), and hydrates were added to produce the desired concentration of OH-ions. The concentration of sodium chlorid employed is not detrimental to the growth of citrus seedlings in the time involved in these experiments.

TABLE 8

COMPOSITION OF NUTRIENT SOLUTION TO WHICH 1000 p.p.m. NaCl HAD BEEN ADDED

Parts per million										
Na	K	Ca	Mg	Fe	Mn	Cl	NO <sub>3</sub>	SO <sub>4</sub>	PO <sub>4</sub>	Total concentration
400	185	159	54	1	0.1	617	718	216	105	2455.1



Fig. 8. Rough-lemon seedlings grown in water cultures maintained at different hydrogen-ion concentrations. Numbers indicate  $P_H$  values maintained. Plants in upper row were taken from cultures in which calcium hydrate was used to maintain  $P_H$  values above 6.0; in lower row sodium hydrate was used.

In the first experiment (series FF, table 9), begun on March 14, rough-lemon seedlings were placed in four solutions of  $P_H$  6, 7, 8, and 9. The  $P_H$  at which it was possible to maintain the original solution for a few hours with no additions of acid or alkali ranged between 5.5 and 6.0 but usually was close to  $P_H$  6, and we have here designated this solution as  $P_H$  6. The three higher  $P_H$  values were maintained by the frequent addition of calcium hydrate. The second experiment (series GG, table 9) was begun March 22, using seedlings from the







same lot as those used in the first. Here the three higher  $P_H$  values were obtained by the addition of sodium hydrate. On April 14 the seedlings were photographed, harvested and prepared for analysis.

Figure 8 shows the growth in the two series. The former seedlings were grown 31 days and were therefore larger than the latter which were grown 23 days. The seedlings grown in culture solutions maintained at  $P_H$  7, 8, and 9 showed marked improvement over those grown at  $P_H$  6.

The seedlings grown at  $P_H$  6 although they grew slowly, made practically the same type of growth as that simultaneously obtained with unmodified Hoagland's solution. The seedlings put out one or two pairs of new leaves which had a good color and were vigorous in every respect. The greatest growth of tops was obtained at  $P_H$  8 in both sets. The tops of seedlings at  $P_H$  8 were superior to those at  $P_H$  6, but the differences between those at  $P_H$  6, 7, and 9 are not significant. The greatest contrast was in the growth of the root systems. In the  $\text{Ca}(\text{OH})_2$  series the roots developed richly branching systems at  $P_H$  7 and 8, but at  $P_H$  9 the growth was more limited. In the  $\text{NaOH}$  series the roots were superior at the higher  $P_H$  values to those in the culture at  $P_H$  6.

Table 9 shows the response of the rough-lemon seedlings to the several culture solutions used. The data represent the weight of seedlings and their composition, in grams per 100 seedlings. The initial composition of seedlings is shown in the last column in the table and serves as a means of judging the amount of absorption which occurred.

The weights and photographs show that the plants made good growth in all cases, yet the higher hydroxyl-ion concentration appears to have stimulated growth. In series FF part of the increased growth at  $P_H$  8.0 might have been due to the increased amount of calcium present, but this explanation does not apply to series GG.

The increased growth of seedlings in solutions above  $P_H$  6.0 was generally accompanied by an increase in the amounts of various ions absorbed. It appears that plants grown at  $P_H$  7.0 and 8.0 absorbed slightly greater amounts of sodium than those grown at  $P_H$  6 in both series, while those at  $P_H$  9.0 showed a very small increase. In series FF the plants at  $P_H$  7.0 absorbed twice as much chlorin as those grown at  $P_H$  6.0. At  $P_H$  8.0 there was a still greater absorption, but at  $P_H$  9.0 it was less than at  $P_H$  7.0. In series GG similar amounts of chlorin were absorbed at  $P_H$  6.0 and 7.0, with an increase at  $P_H$  8.0 and 9.0.

The net absorption of sodium and chlorin for the 31-day period is represented by graphs in diagram 2, together with the ash and the green weight per 100 plants. The data show that these seedlings absorbed a larger quantity of both anions and cations from neutral or alkaline solutions than from the slightly acid solution, and made a correspondingly greater growth.

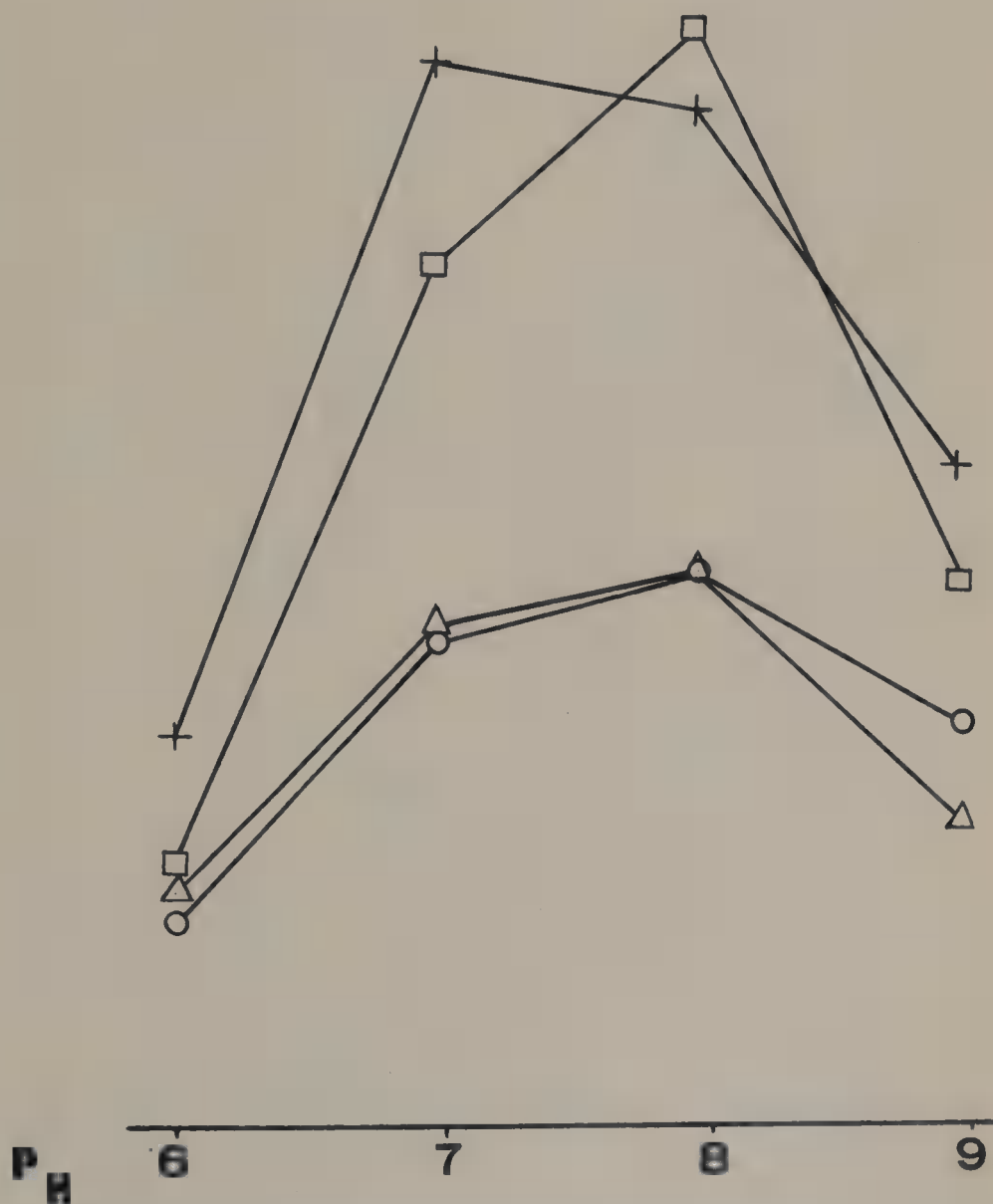


Diagram 2. Growth and absorption of rough-lemon seedlings in nutrient solutions of different  $P_H$  values. □, green weight of plants  $\div 200$ ; +, weight of ash  $\div 5$ ;  $\Delta$ , sodium; O, chlorin.

Table 10 shows the composition of the tops and roots of the same seedlings, expressed as percentages of the ash. The percentage of ash in the tops and roots of both series was greatest when grown in solutions with  $P_H$  values of 7.0 and 8.0. It should be noted that in this case, and in some others the percentages were greater at  $P_H$  9.0 than at  $P_H$  6.0.

The percentage of cations in the ash (table 10) shows little evidence for the idea that plants absorb relatively more cations from alkaline solutions. The tops and roots of series FF showed no significant differences in the percentage of sodium at different  $P_H$  values. In series GG the plants from the solutions of higher  $P_H$  values were in five cases out of six somewhat richer in sodium. It will be remembered, however, that the  $P_H$  of these solutions was raised by the addition of NaOH, and that their sodium content was somewhat increased.

With the possible exception of the tops of series GG kept at  $P_H$  8, the percentage of calcium showed a marked increase when the plants were grown in solutions of  $P_H$  7.0. This fact is more noteworthy when we remember that the higher  $P_H$  values in series FF were produced by the addition of  $\text{Ca}(\text{OH})_2$ .

The percentage of chlorin in the ash of the tops is smaller in the plants grown in the solution having an acid reaction than in those grown in neutral or alkaline solutions. The smallest percentage of chlorin in both series was found in the roots which grew in the neutral solution, while the percentages in other solutions were quite uniform.

With the exception of the roots in series FF the percentage of sulfate was greatest in plants grown at  $P_H$  7.0 or 8.0. In series GG the percentages of sulfate in the ash of tops and roots were approximately the same at  $P_H$  6.0 and  $P_H$  9.0.

The results presented in this paper open a field in which profitable work may be done with other species of plants and in other ranges of hydrogen and hydroxyl-ion concentrations. It is more than probable that acid or alkaline conditions affect, not only the equilibria between the components of the soil, but the equilibrium between the soil solution and the plant.

Some of Theron's results<sup>25</sup> indicate that the amount of growth and the kind of plants used as well as the ratio of certain ions in the solution may be factors affecting the absorption of the various ions at the different  $P_H$  values. The evidence previously published indicates that optimum culture solutions for plants generally have a  $P_H$  value of less

TABLE 10  
COMPOSITION OF ROUGH-LEMON SEEDLINGS GROWN IN HOAGLAND'S SOLUTION + 1000 p.p.m. NaCl AT VARIOUS P<sub>H</sub> VALUES

Series	FF				GG			
	Ca(OH) <sub>2</sub> used to raise P <sub>H</sub> above 6.0 Seedlings 31 days old				NaOH used to raise P <sub>H</sub> above 6.0 Seedlings 23 days old			
	6.0	7.0	8.0	9.0	6.0	7.0	8.0	9.0
P <sub>H</sub> of culture solutions.....								
Per cent ash in dry matter.....	9.08	12.56	12.16	11.30	9.06	12.90	12.83	11.37
Na.....	12.37	12.73	11.51	11.09	8.85	10.06	8.00	11.78
K.....	18.74	17.78	17.99	17.50	19.96	19.89	19.72	18.36
Ca.....	7.37	11.41	9.16	9.09	7.65	8.68	9.14	6.26
Mg.....	2.34	2.30	1.93	1.72	2.31	2.37	2.25	2.14
Cl.....	6.52	8.99	7.88	8.99	5.14	5.55	5.31	8.07
SO <sub>4</sub> .....	2.68	4.45	3.94	2.52	2.90	3.93	3.16	3.05
PO <sub>4</sub> .....	12.31	10.93	10.13	8.28	14.78	13.51	11.01	9.49
Per cent ash in dry matter.....	9.69	20.90	14.22	12.44	9.93	14.49	13.18	10.91
Na.....	7.90	4.11	9.53	6.48	7.47	13.02	11.52	9.46
K.....	32.77	19.27	32.00	31.15	31.93	28.97	35.35	35.59
Ca.....	3.33	12.55	4.92	9.93	3.58	7.63	2.92	3.74
Mg.....	2.13	1.60	2.02	2.53	2.40	2.12	2.00	2.76
Cl.....	12.37	7.48	12.20	12.24	12.11	9.90	13.63	12.42
SO <sub>4</sub> .....	14.07	9.74	13.52	8.00	11.59	14.90	14.90	12.42
PO <sub>4</sub> .....	21.22	20.75	15.67	7.11	21.40	23.54	8.10	9.23

than 7.0, and that OH ions are more harmful than equivalent concentrations of H ions. It is worth noting that the previous experimental work was largely done with seedlings of cereals or legumes, and it is not surprising to find that plants of another genus may react differently. Within a certain range of  $P_H$  values a given concentration of OH ions was less harmful to rough-lemon seedlings than an equivalent concentration of H ions. The graphs in diagram 2 show a certain consistency between green weight, ash, sodium, and chlorin content at different  $P_H$  values. These data, together with the appearance of the plants, indicate that conditions for absorption and growth were definitely superior in the solution of  $P_H$  8.0.

*b. Walnut seedlings.*

Walnut seedling cultures were made in 40-liter jars containing Hoagland's solution to which 1000 p.p.m. NaCl was added. The higher  $P_H$  values in one set were maintained by the addition of NaOH, and in the other by  $\text{Ca}(\text{OH})_2$ . The solutions were changed at intervals of 10 to 14 days and the reactions were adjusted thrice daily. Twelve seedlings were put into each solution on March 27 and allowed to grow until May 9. The plants in all cultures made uniformly good growth as shown by the fresh weights of plants (table 11).

The ash of tops and roots of plants from the different solutions had a rather uniform Cl content, although the roots were uniformly richer in Cl than the tops. It would appear from these figures that the H-ion concentration of the media had no appreciable effect either upon the growth of the plants or upon their chlorin absorption.

The sap was pressed from the tops and roots of three plants from each culture. The  $P_H$ , as determined by the use of the hydrogen electrode (table 11), shows no significant differences. Hoagland and Davis<sup>6</sup> found a similar constancy of  $P_H$  in the sap of *Nitella* from solutions whose  $P_H$  ranged from 5.0 to 9.0.

The analysis of the ash of the walnut seedlings also showed remarkable constancy of composition in most cases. Hoagland<sup>5</sup> reports similarly that there was no general relation between the reaction of the plant sap and the nature of the ion which the plant had absorbed in excess. There is no evidence in these figures for the earlier, and frequently repeated, idea that Na and K are to a certain extent interchangeable in the plant. The percentages of K and Mg showed no significant variations corresponding to the  $P_H$  of the culture solutions.



The idea that proteins may combine with anions or cations, according to the hydrogen-ion concentration of the solution, has led some workers to assume that acid solutions bathing plant roots favor anion absorption and that alkaline solutions favor cation absorption. Our

TABLE 11  
EFFECT OF P<sub>H</sub> OF CULTURE SOLUTION UPON GROWTH AND ABSORPTION BY WALNUT SEEDLINGS

P <sub>H</sub> of culture solution	Series HH							
	6.0 to 6.5		7.0		8.0		9.0	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Fresh weight per 10 plants (grams).....	93	110						
NaOH series.....			80	95	95	123	81	107
Ca(OH) <sub>2</sub> series.....			78	121	93	102	70	106
P <sub>H</sub> of the sap of the plants.....	5.31	5.68						
NaOH series.....			5.48	5.83	5.31	5.82	5.31	5.87
Ca(OH) <sub>2</sub> series.....			5.36	5.75	5.43	5.75	5.26	5.77
Per cent Na in the ash....	8.72	17.03						
NaOH series.....			9.93	20.81	10.64	21.30	10.45	19.96
Ca(OH) <sub>2</sub> series.....			10.22	15.21	8.53	17.13	9.65	16.77
Per cent K in the ash....	27.77	21.87						
NaOH series.....			30.34	19.82	30.61	20.16	33.07	18.77
Ca(OH) <sub>2</sub> series.....			29.80	21.79	29.59	23.81	31.60	21.01
Per cent Ca in the ash....	11.18	7.36						
NaOH series.....			9.76	7.87	9.23	6.42	7.08	7.01
Ca(OH) <sub>2</sub> series.....			10.90	9.05	12.00	7.44	10.27	8.48
Per cent Mg in the ash...	5.24	2.44						
NaOH series.....			5.05	1.62	5.03	2.56	5.01	2.67
Ca(OH) <sub>2</sub> series.....			5.15	2.55	4.90	2.49	4.81	2.60
Per cent Cl in ash of plants.....	4.98	8.10						
NaOH series.....			4.87	7.45	4.92	7.78	4.96	8.97
Ca(OH) <sub>2</sub> series.....			4.04	7.20	3.36	8.15	4.00	8.71

results, however, seem to indicate that the neutral point (P<sub>H</sub> 7.0) is not a point of great importance so far as absorption by plants is concerned. In other words, there is no evident reason for assuming that the neutral point of distilled water is a sort of turning point, or isoelectric point, in regard to absorption of ions from a solution by plant roots.

# VIII. THE EFFECTS OF SODIUM CHLORID ON ORANGE TREES IN SOIL, AND THE RESULTS OF LEACHING

When citrus trees are grown in soils containing harmful amounts of saline material, the leaves are often the first organs to show injury. For a time the leaves appear yellowish, then the margins and apical regions die, and finally an excessive abscission of leaves occurs which makes the unhealthy condition of the trees very conspicuous. Loughridge<sup>12</sup> and Hilgard<sup>3</sup> were among the first to emphasize the toxicity of

TABLE 12

VOLUME OF LIQUIDS APPLIED TO THE CULTURES, AND TRANSPIRATION OF  
ORANGE TREES

(Figures in parenthesis were not included in making averages)

Tree	Volume of liquids added (liters)					Drainage water (liters)	Trans- piration (liters)	Ratio of transpira- tion to dry weight of tree
	Nutrient solution	NaCl solution	Distilled water	Tap water	Total			
84	60	.....	81	274	415	0	415	436
85	(30)	.....	(77)	(146)	(253)	0	(253)	(357)
86	63	.....	93	304	460	0	460	465
87	60	.....	103	328	491	0	491	.....
88	60	.....	104	306	470	0	470	381
89	69	40	33	58	200	46	154	.....
90	69	42	39	173	323	31	292	603
91	66	39	45	212	362	31	331	519
92	66	24	37	204	331	31	300	472

sodium chlorid to citrus trees. More recently Kelley and Thomas<sup>9</sup> have added to our knowledge of the effects of saline irrigation water. The present writers<sup>18</sup> have described the results produced experimentally when young orange trees were grown in sand cultures to which solutions of sodium chlorid were regularly added. We have noted that the chlorophyll of the leaves had a tendency to fade, and that premature abscission was the rule, except where sufficient amounts of calcium salts were present. Twigs and roots were restricted in their growth and eventually many were killed. Rudolfs<sup>24</sup> has reported similar effects of sodium chlorid on other species of trees.

In the following pages we give additional data on the effects experimentally produced by the addition of sodium chlorid to sand and to soil cultures, with especial reference to the chlorid content of orange

leaves, and to the changes which occurred when the soil impregnated with salt was leached with water. The importance of the results lies largely in the fact that we have here produced definite symptoms by the application of a known cause.

#### *A. Trees under controlled conditions.*

Young orange (*Citrus sinensis*) trees were grown in large galvanized-iron cans containing sand or soil under conditions which permitted of simple and convenient observation. The details of the general cultural methods have been given elsewhere.<sup>17</sup> The trees were planted May 20, 1920, and removed in February, 1922.

##### *1. Sodium chlorid added to non-saline soil.*

Trees 13 and 16 were grown in silica sand which received nutrient solution plus 1000 parts per million of sodium chlorid. The other trees were grown in cans which contained soil taken from an uncultivated area on the Citrus Experiment Station property. The soil has been classified as Sierra loam and occurs in regions devoted to citrus culture in the vicinity of Riverside.

Trees 84-88 grew in the soil described above and were irrigated for the first eleven months with distilled water. Trees 89-92 were grown in soil from the same source and were irrigated for the same period with a solution containing 1500 parts per million of sodium chlorid. The latter were seriously injured and showed symptoms which will be described later. During the first eleven months there was no drainage water from the soil. In the latter part of April, 1921, cultures 89-92 were leached with tap water until, as shown in table 12, 30 to 46 liters had been recovered from each container. From that time nutrient solution was added to all cultures to furnish the trees a more nearly optimum supply of nutrient ions.

##### *(a) Changes in the soil.*

When the soil from cultures irrigated for five months with distilled water was sampled on October 8, the amount of material extracted with distilled water (table 13) was about half that obtained five months earlier. The concentration of  $\text{HCO}_3$  ions was much diminished and the  $\text{NO}_3$  concentration increased from nothing to an appreciable amount. The concentrations of  $\text{SO}_4$  and of Cl anions showed no significant change.

The solutes extractable with water eleven months after installing the cultures showed slight differences in concentration from those observed at the expiration of five months. The soil of cans 89-92 gave extracts which contained more Ca, Mg, Na, and Cl than that from cultures receiving distilled water. At the end of eleven months their total content of soluble solids had increased from 589 to 1033 parts per million but the  $P_H$  of the extract showed little change.

TABLE 13

ANALYSIS OF SOIL USED IN EXPERIMENTS ON THE EFFECT OF SODIUM CHLORIDE  
(Calculated on the basis of air-dry soil)

	Samples taken when experiment was installed	Cultures 84-88, which received distilled water		Cultures 89-92, which received 1500 p.p.m. sodium chlorid	
	May 20, 1920	Oct. 8, 1920	Apr. 14, 1921	Oct. 8, 1920	Apr. 14, 1921
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
SiO <sub>2</sub> .....	.....	27	26	25	42
Ca.....	.....	28	24	53	91
Mg.....	.....	9	9	16	28
Na.....	.....	13	22	107	191
HCO <sub>3</sub> .....	183	64	101	61	115
CO <sub>3</sub> .....	0				
Cl.....	18	24	28	221	436
NO <sub>3</sub> .....	0	66	11	58	48
SO <sub>4</sub> .....	22	19	10	15	33
PO <sub>4</sub> .....	18				
Total solids as sulphates...	400	178	194	589	1033
$P_H$ .....	.....	6.7	6.8	6.7	7.0

The soil in cultures 89-92 was leached with distilled water on April 15, 1921, until percolates began to appear. Only the first portion of the percolate was alkaline to phenolphthalein, although subsequent portions were alkaline to other indicators. A filtered sample of the first 500 c.c. of percolate from culture 92 showed 12 parts per million CO<sub>3</sub> and 67 parts per million of HCO<sub>3</sub>. On the following day the percolates obtained from cans 89 and 92 were still alkaline to phenolphthalein. The application of additional quantities of distilled water followed by nutrient solution finally gave percolates which had  $P_H$  values below 8.3.

The first portions of the percolates from cans 87 and 88 showed no alkalinity to phenolphthalein. The results of this and subsequent determinations are shown in table 14. The third column of table 14 contains the  $P_H$  values of the percolates obtained at the conclusion of the leaching process which had been in progress for three weeks.

The concentration of chlorin in the percolate varied considerably from time to time. The chlorin content of the percolate from can 92 was small, more or less in keeping with the relative amount of sodium chlorid added. The point of interest was the relatively extensive and the long continued formation of black alkali in the soil of this container in comparison with the soils of cans 90 and 91, which received considerably larger amounts of the salt.

TABLE 14  
DATA ON THE PERCOLATES FROM VARIOUS CULTURES

Culture	Grams of NaCl added	$P_H$ of percolates after continued leaching 21 days	Parts per million of chlorid in percolates		
			After 1 day	After 6 days	After 15 days
87	0.0	.....	25	.....	.....
88	0.0	.....	21	.....	.....
89	59.3	7.8	780	984	1179
90	63.4	6.8	255	1167	701
91	58.8	7.0	280	1186	423
92	35.5	7.8	248	44	199

Nutrient solution was added from time to time during the leaching process as well as afterward, and both distilled and tap water were used to supply the soil moisture after the salt treatment was discontinued.

(b) *Effects on the trees.*

The trees in cultures 84-88, which received only distilled water, grew fairly well for some time, but eventually showed the symptoms commonly observed when the supply of nitrates is insufficient. After a lapse of several months, the soils were irrigated from time to time with Hoagland's nutrient solution. The effect of the nutrient solution was shown in the improved appearance of the trees and in their increased growth. When the trees were removed from the cultures their condition was good and the weight of the various parts (table 15) exceeded that of trees grown simultaneously in sand receiving continuous applications of nutrient solution.



The trees in cultures 89-92 grew only a short time before they began to show the harmful effects of the sodium chlorid in the irrigation water. The leaves eventually became yellow and showed dead margins and tips which are characteristic of salt injury (fig. 9). These leaves fell after a time and were followed by a new crop which usually showed more acute symptoms of injury. The symptoms known as "mottle-leaf" did not appear on any of the trees receiving saline irrigation water. We may note in passing that the percolate from these

TABLE 15  
DATA ON THE TREES GROWN IN SOIL CULTURES  
(Figures in parenthesis not included in the averages)

Tree	Number of leaves on tree at conclusion of experiment	Dry weight (grams)					
		Leaves	Shoots	Trunk	Root	Rootlets	Total
84.....	995	224	132	171	197	227	951
85.....	(1042)	(206)	(96)	(141)	(135)	(130)	(708)
86.....	1172	311	126	234	184	234	989
87.....	(873)		(148)	(293)	(256)	(239)	
88.....	730	194	152	368	316	204	1234
Average.....	966	210	137	258	232	222	1058
89.....	(473)		(36)	(159)	(103)	(39)	
90.....	464	117	75	123	94	75	484
91.....	707	153	95	139	136	115	638
92.....	623	139	89	170	136	101	635
Average.....	598	136	86	144	122	97	586

cultures at the time when the injury was very severe contained 91 parts per million of calcium (table 13), which may have been responsible for the absence of mottle-leaf on these trees, although it was not sufficient to prevent tip burn. Cummins and Kelley<sup>1</sup> have reported that sodium salts, when applied to soil, set free calcium, and further applications of sodium salts brought about an excessive concentration of soluble sodium in the soil solution, and eventually the soil became deflocculated and impervious.

The depauperate leaves shown in fig. 9 are quite representative of the effects of saline irrigation water on these trees. None of them attained the size usually reached by leaves of the Valencia orange tree. The tissue on one side of the midrib frequently made less growth than

that on the other, with the resulting formation of asymmetrical leaves. In February, 1921 (two months prior to making the photograph), the leaves of trees 90 and 91 showed a considerable amount of tip burn and the older leaves were falling rapidly. Trees 89-92 produced a large number of flowers, in this respect resembling unhealthy trees in the field. The bark on the trunks of trees 89-92 was killed in areas near the surface of the soil. The dead areas were above the surface of the soil and could not have been due to contact with the saline



Fig. 9. Orange leaves from tree which was injured by applications of 1500 p.p.m. sodium chlorid.

irrigation water, since special care was used to prevent it from coming in contact with the trunks. The trunks of trees 84-88 were free from this type of injury. The shoot and root growth of trees 89-92 was notably limited.

In April, 1921, the soil of cans 89 to 92 was leached with distilled water. Leaching of the soil and removal of the drainage water were carried on while the trees were *in situ*, hence any ill effects due to saturation of the soil during the leaching process should have been evident. The leaching process greatly benefited the trees, as will be shown subsequently.

Shortly after the first drainage water was obtained, finely divided colloidal material appeared in it and the rate of percolation was

greatly reduced. Hoagland's nutrient solution was then added in small amounts from time to time, care being taken not to have an excess of solution standing for any long time on the surface of the soil. Frequent additions of solution and removals of drainage water were made, the method followed being in imitation of the removal of salts from precipitates on a filter.

The leaching process naturally removed salts which would have been useful to the trees, and the addition of nutrient solution was therefore advisable if the series were to be at all comparable with that which received no sodium chlorid. The total volume of nutrient solution applied to the soils in series 89-92 was somewhat in excess of that given to the other series (table 12), nevertheless it did not produce in the ten months during which no sodium chlorid was

TABLE 16  
P<sub>H</sub> AND OSMOTIC PRESSURE OF THE SAP OF MATURE LEAVES

Series	P <sub>H</sub>	Osmotic pressure (average of 2 determinations)
84-88.....	5.80	21.03
89-92.....	5.82	20.32

applied, trees equal to those produced in the other series. The dry weight of the trees was only about half that of the trees grown in the soil to which no sodium chlorid was added.

Determinations of the hydrogen-ion concentration and of the osmotic pressure of the sap of mature leaves were made May 26, 1921. The former was electrometrically determined and the latter was computed from the lowering of the freezing point. Table 16 shows no significant differences in the P<sub>H</sub> of the leaf sap, though as we have seen, the roots of the trees were growing in a soil which had received large concentrations of saline irrigation water and which upon being leached had shown black alkali in the drainage water. Rudolfs<sup>24</sup> has expressed the belief that chlorin increases the acidity in the plant cell, accelerating or harming the vital activities according to the amount employed, but the above results indicate that the chlorin had no appreciable effect on the P<sub>H</sub> of the plant sap. A large salt content in leaves may tend to keep a given P<sub>H</sub> more constant rather than to change the P<sub>H</sub> value.



The trees in both series made very satisfactory growth during the summer of 1921. The appearance of the trees on August 25 may be seen in fig. 11, which shows material improvement over the condition four months earlier (fig. 10). The new foliage produced in series 89-92 was free from the unfavorable characters shown previously.



Fig. 10. Trees in the experiment described. From left to right; trees 87, 88, 89, 90, and 91. Trees 87 and 88 grew eleven months in soil which received distilled water. The other trees grew in similar soil and received water containing 1500 parts per million of sodium chlorid.



Fig. 11. Trees in the experiment at the end of sixteen months. From left to right; trees 88, 89, 90, 91, and 92. The four trees on the right show recovery from the injury induced by the application of sodium chlorid to the soil, and resemble the typical control tree 88 (on the left).

The difference between the volume of water (or solution) added and that of the percolate has been reckoned as transpiration (table 12). While there may be some error in this procedure, it is common to all the trees. The amount of water lost by direct evaporation from the cans was small in comparison with that transpired by the foliage of

the trees. The ratio of transpiration to dry weight is somewhat greater in the case of the less thrifty trees in series 89-92. Data in table 16 show that the osmotic pressure of the sap of the leaves of the control series was not significantly greater than that of the sap of the injured trees at the time when leaching began.

(c) *The chlorin content of the leaves.*

Broadly speaking we may say that the addition of sodium chlorid to the soil produced no significant changes in the composition of the ash of the leaves except in respect to their sodium and chlorin content (table 17). Without further evidence we are not justified in assuming that there was a substitution of sodium for potassium.

TABLE 17

THE EFFECT OF SODIUM CHLORID UPON THE COMPOSITION OF ORANGE LEAVES  
(Expressed as a percentage of the ash)

	Sand cultures	Soil cultures		
	Trees 13-16	Trees 84-88	Trees 89-92	
	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Na in culture solution.....	400	0	590	590
Cl in culture solution.....	898	0	910	910
Condition of leaves.....	No injury.	No injury.	Injured.	Injured, about to fall.
Constituents of the ash:				
Na.....	1.61	1.64	4.38	3.98
K.....	38.25	8.82	9.86	8.85
Ca.....	8.77	29.77	28.55	26.85
Mg.....	1.15	2.36	2.78	2.65
Cl.....	4.73	0.28	17.03	21.36
SO <sub>4</sub> .....		2.36	2.27	2.55
PO <sub>4</sub> .....		3.71	5.26	3.88
Ash expressed as a per cent of dry matter.....	16.82	14.78	17.54	17.19

The ratio of soluble to insoluble constituents in the leaves was determined by extracting the dry-ground-leaf material with an excess of distilled water for several hours, filtering, and analyzing the soluble and insoluble portions separately. The ratio between the soluble and insoluble portions of each constituent is given in table 18. In the



case of the individual ions there is a significant difference in Na and PO<sub>4</sub> and a puzzling difference in the case of SO<sub>4</sub>. The others show no more variation than we are accustomed to find in this kind of work. The proportion of soluble PO<sub>4</sub> in the dried material bears a sort of inverse relationship to the total amounts present, although further evidence is necessary before we can attach much significance to these values.

TABLE 18  
THE RELATIVE SOLUBILITY OF THE ASH CONSTITUENTS OF VALENCIA ORANGE LEAVES  
GROWN IN SOIL. FIGURES REPRESENT PERCENTAGE OF THE TOTAL  
AMOUNT FOUND.

Source of leaves	Trees 84-88	Trees 89-92	
Condition of leaves	No injury	Injured	Injured, about to fall
	Soluble	Soluble	Soluble
Ash.....	67.51	70.80	71.15
Na.....	67.82	74.92	90.97
K.....	94.48	93.12	97.84
Ca.....	61.23	62.38	61.85
Mg.....	92.76	93.17	94.58
Cl.....	100.00	100.00	99.87
SO <sub>4</sub> .....	75.86	81.44	62.23
PO <sub>4</sub> .....	73.90	54.90	60.53

The percentage of soluble Cl is of great importance in view of the greatly increased amounts of this element which the injured leaves contained. The fact that all the Cl in the dried leaf material was soluble, gives some idea of the cause of the increasing deleterious effects which are associated with the absorption of chlorids. There is no evidence that the chlorin ions migrate from the leaves before abscission.

2. Soil previously made saline by orchard irrigation.

Valencia orange trees were also planted in cultures like those previously described to test the possibility of removing excessive amounts of salts under controlled conditions. The soil\* was obtained from an orange grove which had been injured by saline irrigation water of the following composition:

Ca	Mg	Na	Cl	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	NO <sub>3</sub>	SiO <sub>2</sub>	Total solids as sulphates
172	58	228	627	0	213	76	28	39	1578

\* The soil on which the grove stands has been classified by the Bureau of Soils as Ramona loam.

When the soil samples were obtained from the grove we noted that practically all the functioning roots were found between 12 and 24 inches from the surface. Samples of the first 12 inches and of the second 12 inches were collected, each lot being kept separate. Sixteen large galvanized-iron cans were filled with the soil: five cans with the first foot layer, five with the second foot layer, and six with a mixture of equal parts of the two layers. A Valencia orange tree was planted in each can May 21, 1920. Three of the cans in each

TABLE 19

COMPOSITION OF THE WATER EXTRACTS OF SALINE SOIL USED IN CULTURES FOUR MONTHS AFTER THE EXPERIMENT WAS BEGUN, EXPRESSED AS PARTS PER MILLION OF AIR-DRY SOIL

	First foot		Second foot		Mixture of first and second foot	
	Unleached	Leached	Unleached	Leached	Unleached	Leached
Ca.....	325	95	15	8	132	50
Mg.....	100	34	5	2	42	20
Na.....	438	247	207	87	340	247
Cl.....	1002	390	200	89	566	334
CO <sub>3</sub> .....	0	0	0	0	0	0
HCO <sub>3</sub> .....	107	122	115	137	82	112
SO <sub>4</sub> .....	227	97	52	37	122	90
NO <sub>3</sub> .....	361	156	47	11	221	130
SiO <sub>2</sub> .....	46	48	56	45	45	45
Total solids as sulphates...	2950	1251	714	420	1710	1030
P <sub>H</sub> .....	7.0	7.0	7.0	6.8	6.9	7.0
Tap water added (liters)....	12.8	40.0	14.3	40.0	13.0	40.0
Drainage recovered (liters)	0	23.9	0	13.0	0	15.3

set were leached and the others were kept as near the optimum water content as possible. The amounts of soluble salt in the soil and the effect of leaching are shown by data in table 19. The total quantities of water and solution applied to the leached soils were about four times those given to the unleached soils. Analyses of the percolates showed a rather constant fall in chlorid content during the first year. Because of the effects of the leaching process upon the soil, we therefore applied a culture solution from time to time.

In a general way the growth of the trees in the several cultures reflected the content of soluble matter in the soils. The trees in the unleached first-foot soil were short lived. They sent out shoots which

TABLE 20  
VOLUME OF LIQUIDS ADDED AND GROWTH OF ORANGE TREES IN CULTURES IN SALINE SOIL

	Water applied	Nutrient solution applied	Average number of leaves on tree	Average dry weight per tree (grams)					
				Leaves	Shoots	Trunks	Roots	Rootlets	Total
	<i>Liters</i>	<i>Liters</i>							
First foot, leached <sup>1</sup> .....	268	57	704	123	77	145	127	110	582
Second foot, unleached.....	132	0	279	26	35	116	79	28	284
Second foot, leached.....	419	63	845	127	91	218	161	193	790
Mixture of first and second foot, unleached <sup>1</sup> .....	124	0	326	48	30	106	85	37	306
Mixture of first and second foot, leached <sup>2</sup> .....	360	60	754	143	96	128	123	195	685

<sup>1</sup> Two of the three trees in the set died and new trees were installed four months after the experiment was begun.  
<sup>2</sup> One of the three trees in the set failed to start growth and was replaced by a new tree four months after the experiment was begun.

reached a length of one or two inches, but failed to develop further. The leaves died before reaching their usual size; a little later the new shoots also died. No recovery occurred, in spite of careful attention. The trees in the other unleached soil made restricted growth, and showed marked evidence of salt injury quite similar to that observed where sodium chlorid was added. The leaves were small, yellowish, and dead at the tips. No tendency to mottle-leaf was observed in any of these trees. Table 20 gives the average dry weights of the

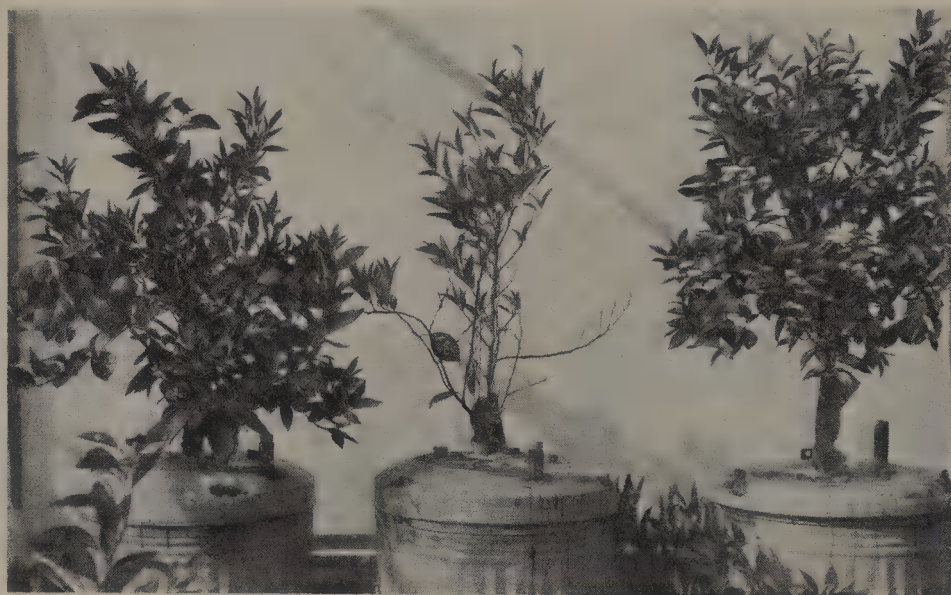


Fig. 12. Trees grown in leached and unleached saline soil. Tree in center grown in unleached second-foot soil from an orange grove; other two trees grown in leached second-foot soil.

trees at the end of the experiment, and represents the relative growth made by the several series. The best growth was produced in the second-foot soil. Figure 12 shows certain trees in the leached and unleached soils just prior to the termination of the experiment in December, 1922. The amounts of chlorin in the ash of certain trees are given in table 21, and supplement the data given in table 17.

TABLE 21

PERCENTAGE OF CHLORIN IN THE ASH OF ORANGE TREES GROWN IN UNLEACHED SALINE SOIL

	Leaves	Shoots	Trunks	Roots	Rootlets
First foot, unleached.....			4.45	11.99	
Second foot, unleached.....	11.79	2.71	1.98	3.28	3.58

*B. Trees grown in the field.*

Samples of leaves and shoots were collected in April from mature orange trees which had been severely injured by the application of saline irrigation water. For purposes of comparison, samples of leaves and shoots were also taken from healthy trees of the same age in an adjoining grove which had received non-saline irrigation water. The water applied to the injured trees contained 480 p.p.m chlorin

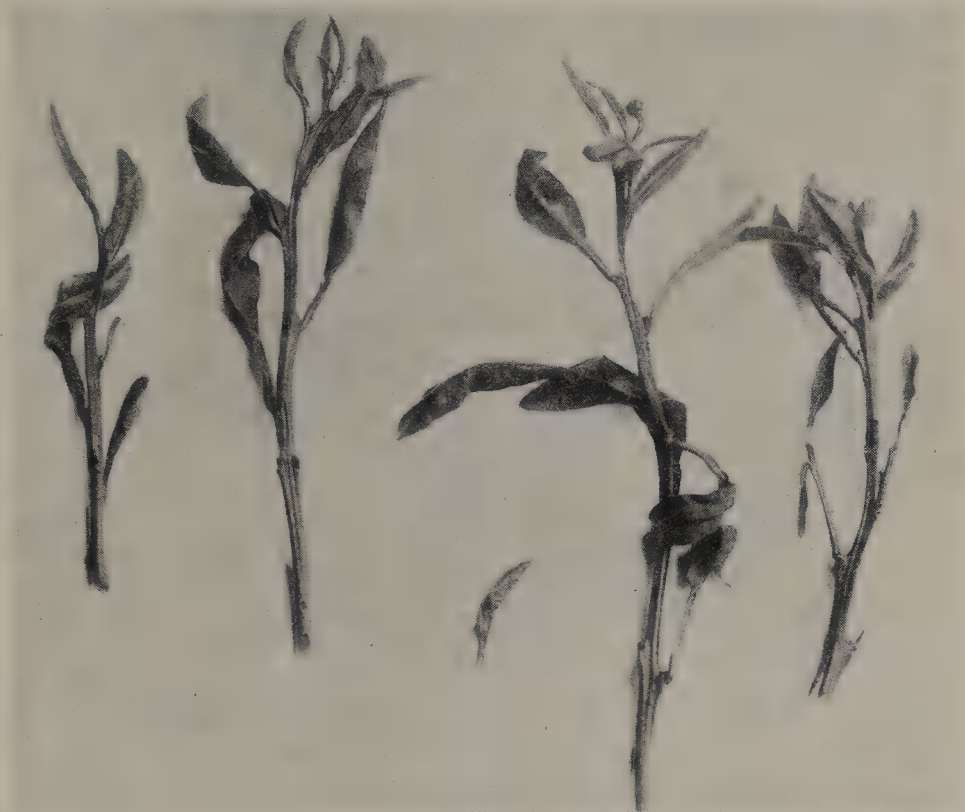


Fig. 13. Young shoots from orange trees in the field showing severe injury following the use of saline irrigation water.

and 200 of sodium. Samples of the soil from this grove were collected at the time the leaves and shoots were taken from the trees. Analyses of the soil samples made by S. M. Brown showed the following amounts of chlorin: 1st foot, 229 p.p.m.; 2nd foot, 211 p.p.m.; 3d foot, 90 p.p.m.; and 4th foot, 99 p.p.m.

The shoots from the injured trees were of two kinds, young and old. There was a region below the tip of each young shoot (fig. 13) which was discolored and shrunken. The older shoots were more or less completely defoliated and were putting out weak, unhealthy secondary shoots, many of which fell after making very feeble growth.



The young leaves were often greatly reduced in size and showed discolored, blistered surfaces (fig. 14). Leaves which reached the usual size often had dead tips or margins. The old leaves which were collected in April had been produced the preceding year and were dead at the tips and margins (fig. 15). At the time they were collected, many of them were falling from the trees. Table 22 shows the



Fig. 14. Young leaves from shoots like those shown in figure 13. The surfaces of these leaves had a blistered appearance.



Fig. 15. Old leaves from orange trees which showed severe injury from saline irrigation water.

composition of the shoots and leaves. The very high percentage of chlorin in the shoots and leaves of the injured trees is one of the conspicuous features of this table. The percentage of chlorin in the young shoots and leaves was greater than that in the older organs. It may be well that only the organs which contained the smaller amounts of chlorin were able to survive. Another interesting feature of the analyses is the small percentage of sodium in the old injured leaves.

TABLE 22

THE COMPOSITION OF SHOOTS AND LEAVES OF ORANGE TREES IRRIGATED WITH SALINE AND NON-SALINE WATER

	Irrigated with saline water					Irrigated with non-saline water	
	Shoots		Leaves			Shoots	Leaves
	Young succulent shoots of the last cycle	Defoliated woody shoots of the preceding cycle	Young leaves showing injury	Old leaves still attached but injured	Old leaves fallen as a result of injury	Woody shoots of the preceding cycle	Old leaves not injured
Constituents of the ash:							
Na.....	5.78	2.12	4.71	1.28	1.19	1.19	2.23
K.....	18.45	5.85	19.41	3.55	2.75	6.92	5.22
Ca.....	18.91	29.00	17.05	30.80	32.10	31.30	32.00
Mg.....	4.52	4.38	3.96	4.02	4.29	2.62	2.23
Cl.....	22.64	7.23	22.70	15.57	14.45	0.14	0.33
SO <sub>4</sub> .....	1.98	4.46	2.40	3.58	3.73	2.43	3.11
PO <sub>4</sub> .....	6.04	5.81	7.83	2.18	1.48	4.48	1.99
Ash expressed as per cent of the dry matter.....	10.59	8.51	10.28	17.64	18.03	4.93	12.65

The percentage of ash in the dry matter of the woody shoots and of the old leaves on the injured trees was considerably greater than that of organs of the same age from healthy trees.

The results of these experiments and analyses extend our knowledge of the effects of sodium chlorid on orange trees. They agree in showing reduced growth of roots, shoots, and leaves. Eventually the trees suffer from premature defoliation. The analyses show that the Na and Cl content of injured shoots is greater than that of healthy shoots.

It is also shown that harmful amounts of sodium chlorid may be leached from a soil and that satisfactory growth conditions may be produced if suitable nutrient salts are added soon after the leaching.

IX. THE EFFECT OF SODIUM BICARBONATE ON YOUNG ORANGE TREES

In the present studies, Valencia orange trees (*Citrus sinensis* Osbeck), were grown in sand cultures to which modified Hoagland's solution containing 1000 p.p.m sodium bicarbonate was added. The technique of the care of the cultures was the same as that previously described. Table 23 shows the concentration of the various ions in the culture solutions and the salts employed. The solution applied to trees 30-35 contained no calcium, while that of the other two series contained 159 p.p.m. The P<sub>H</sub> of the solution applied to trees 30-35 was 7.55, while that of trees 38, 40 and 41 was 7.45. The trees were planted in the containers of sand on May 21, 1920, and were removed on September 20, 1921, having grown during the same period as trees 6-11, 12 and 16, 18-23, and 27 and 28, the data on which have already been reported in previous publications.<sup>17, 18</sup>

TABLE 23  
COMPOSITION OF CULTURE SOLUTIONS EMPLOYED IN EXPERIMENTS ON THE EFFECTS  
OF BICARBONATES ON ORANGE TREES  
(Parts per million)

	Trees		
	1 and 2	30-35	38, 40, 41
Na.....	7	280	280
K.....	185	496	496
Mn.....	0.1	0.1	0.1
Ca.....	159	0	159
Mg.....	54	54	54
Fe.....	1	1	1
Cl.....	10	10	291
NO <sub>3</sub> .....	718	718	718
SO <sub>4</sub> .....	216	214	214
HCO <sub>3</sub> .....		725	725
PO <sub>4</sub> .....	105	105	105
Total concentration.....	1455.1	2603.1	3043.1

Salts employed in making the nutrient solution.	KNO <sub>3</sub>	KNO <sub>3</sub>	KNO <sub>3</sub>
	MgSO <sub>4</sub> +7H <sub>2</sub> O	MgSO <sub>4</sub> +7H <sub>2</sub> O	MgSO <sub>4</sub> +7H <sub>2</sub> O
	NaCl	NaCl	NaCl
	Ca(NO <sub>3</sub> ) <sub>2</sub> +4H <sub>2</sub> O	KNO <sub>3</sub>	KNO <sub>3</sub>
	KH <sub>2</sub> PO <sub>4</sub>	KH <sub>2</sub> PO <sub>4</sub>	KH <sub>2</sub> PO <sub>4</sub>
	MnSO <sub>4</sub>	NaHCO <sub>3</sub>	NaHCO <sub>3</sub>
	Ferric tartrate	MnSO <sub>4</sub>	CaCl <sub>2</sub> +2H <sub>2</sub> O
		Ferric tartrate	MnSO <sub>4</sub>
			Ferric tartrate

TABLE 24  
NUMBER OF LEAVES, DRY WEIGHT OF VARIOUS PORTIONS OF CITRUS TREES AND WATER REQUIREMENT

Tree	Number of leaves	Dry weight (dried at 60°-65° C.) in grams						Total culture solution added	Total distilled water added	Total drainage water	Trans- pira- tion	Water require- ment
		Leaves	Shoots	Trunk	Root	Rootlets	Total					
30.....	52	4.5	7	58	47	9.5	126	Liters 184	Liters 49	Liters 145.5	Liters 87.5	690
31.....	190	27	9.5	134	164	13	347.5	192	61	141.25	111.75	320
32.....	81	8.5	14	92	103	8	225.5	196	47	138.35	104.65	460
33.....	218	22	12.5	70	87	17	208.5	186	55	142.80	98.2	470
34.....	93	19	13	51	55	13.5	151.5	178	41	136.60	82.4	540
35.....	96	15	24	127	133	13	312	186	56	131.1	110.9	360
Average per tree (30-35).....	122	16	13.3	88.7	98.2	12.3	228.5					
38.....	385	54	26	86	84	39.7	289.7	208	93	109.9	191.1	660
40.....	330	53	19.5	86	76	34	268.5	208	75	150.75	132.25	490
41.....	425	84.5	33	80	145	31	373.5	236	75	125.08	185.92	500
Average per tree (38, 40, 41).....	380	63.8	26.2	84	101.7	34.9	310.6					

TABLE 25  
EFFECTS OF SODIUM SALTS ON ORANGE TREES  
Number of leaves, dry weight, and transpiration of average trees of each series

Series	Concentration of ions in addition to that of the control	Number of leaves on average tree	Dry weight (dried at 60°-65° C.) in grams						Transpiration (liters)
			Leaves	Shoots	Trunk	Root	Rootlets	Total	
42-46.....	Ca of Hoagland's solution replaced by K.....	110	19	20.8	85.1	115	18.9	258.3	85.9
6-11.....	393 p.p.m. Na..... 607 p.p.m. Cl.....	97	15.6	12.8	91.7	78.9	12.1	211.2	74
18-23.....	323 p.p.m. Na..... 674 p.p.m. SO <sub>4</sub> .....	53	14.7	14.6	75.2	107.3	21.8	233.5	95.5
30-35.....	273 p.p.m. Na..... 725 p.p.m. HCO <sub>3</sub> .....	122	16	13.3	88.7	98.2	12.3	228.5	99.2
13 and 16.....	393 p.p.m. Na..... 159 p.p.m. Ca..... 888 p.p.m. Cl.....	498	95.2	35.2	104.2	106	46	386.6	126
27 and 28.....	323 p.p.m. Na..... 674 p.p.m. SO <sub>4</sub> ..... 159 p.p.m. Ca..... 281 p.p.m. Cl.....	749	173	86.5	139	132.5	124	655	183.1
38, 40 and 41.....	273 p.p.m. Na..... 725 p.p.m. HCO <sub>3</sub> ..... 159 p.p.m. Ca..... 281 p.p.m. Cl.....	380	63.8	26.2	84	101.7	34.9	310.6	169.8
1.....	Control.....	996	237.1	122.5	194.0	212	136	901.6	228



The data in table 24 show the number of leaves, dry weight, and transpiration of the trees in the two series. Table 25 gives a wider comparison of the composition of the solutions and the average number of leaves, dry weight and transpiration of average trees in these and other series to which sodium salts were added at the rate of 1000 p.p.m. It shows that the growth made by trees 30-35 was similar to that made by the other trees receiving no calcium. The trees grown with the

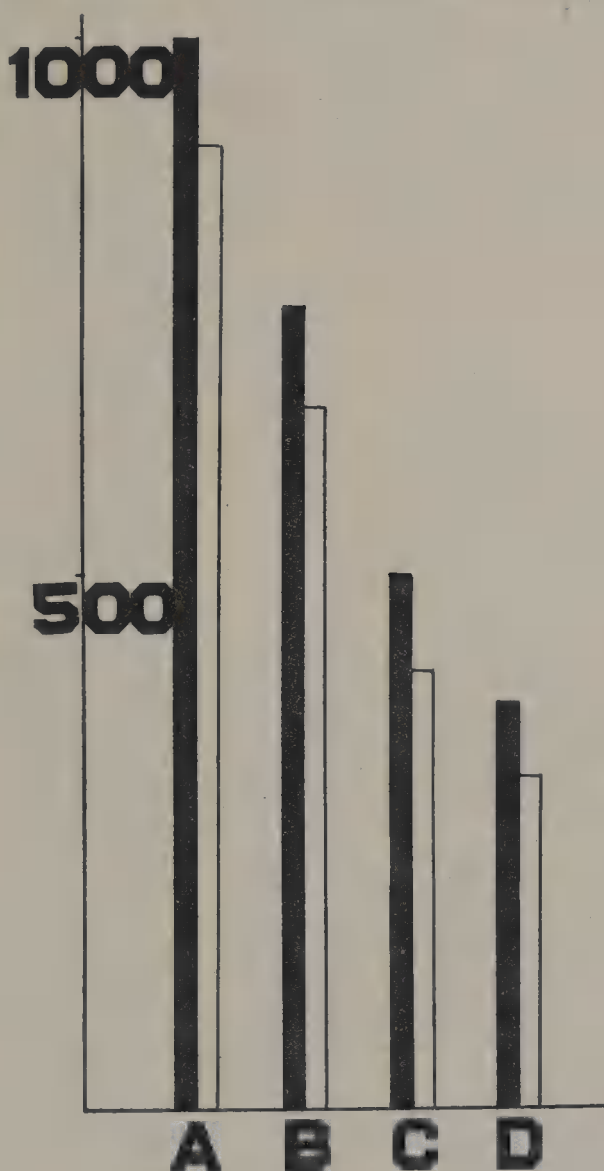


Diagram 3. Comparative effects of three sodium salts upon orange trees. Each salt was added to the culture solution at the rate of 1000 p.p.m. The shaded bars represent number of leaves on the trees, and the unshaded bars represent grams of dry weight of trees. A, complete nutrient solution; B, nutrient solution plus 1000 p.p.m. sodium sulfate; C, nutrient solution plus 1000 p.p.m. sodium chlorid; D, nutrient solution plus 1000 p.p.m. sodium bicarbonate.

additional sulfate (trees 27 and 28) grew better than trees which received the additional chlorin (trees 13 and 16) and these in turn grew better than trees which received bicarbonate (trees 38, 40 and 41). The comparative effects of three sodium salts upon the growth of orange trees are shown in diagram 3.

The effect of bicarbonate on walnut seedlings (table 6) was also more toxic than that of chlorid or sulfate in conjunction with sodium cations.



Fig. 16. Trees 34 and 35 at the end of fifteen months in sand cultures which had received modified nutrient solution lacking calcium and containing 1000 p.p.m. sodium bicarbonate.

Figure 16 shows the tops of trees 34 and 35 on August 25, 1921, after 15 months in cultures which received sodium bicarbonate but no calcium. Many of the leaves had fallen and most of those produced subsequently fell before attaining full size. Many of the shoots became leafless and died. Figure 16 also shows the recurved condition, typical of leaves grown in the absence of calcium (cf. 18, pl. 3, fig. 1). The trunks were still alive when the trees were removed from the containers. The trunk and root system are the last parts of an orange tree to die, under these conditions, due possibly to their retention of calcium and to the slower rate of metabolism in their tissues.



Fig. 17. Trees 38, 40 and 41 at the end of fifteen months in sand cultures which received complete nutrient solution plus 1000 p.p.m. sodium bicarbonate.



Fig. 18. Trees in sand cultures both of which received 1000 p.p.m. sodium bicarbonate. The tree on the left received no calcium, while the tree on the right received 159 p.p.m. calcium as calcium chlorid. The photograph was taken fifteen months after the experiment began.



Figure 17 shows the growth made by the tree tops when sodium bicarbonate was present along with calcium chlorid. The leaves were retained much longer than in the case where calcium was absent from the culture solution. Figure 18 contrasts the condition of the tree tops when calcium is present, with the defoliation that occurs when it is absent. The chlorotic leaves shown in figure 19 are typical of many which appeared on trees 30-35 after a year had passed. These leaves were similar to those which are frequently found on trees in groves where the soil contains bicarbonates and carbonates (Lipman,<sup>11</sup>).

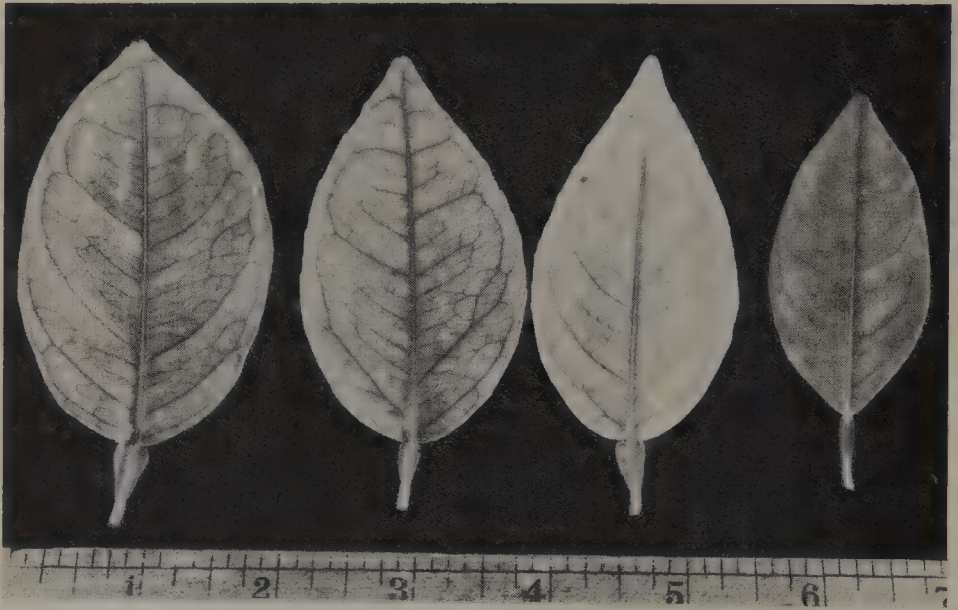


Fig. 19. Chlorotic leaves from trees which received sodium bicarbonate without calcium. Leaves like these never recovered. Scale of inches at the bottom of the picture.

When a condition of chlorosis like that shown by these leaves is attained, no recovery occurs, even though iron is added to the cultures at frequent intervals. The leaves of trees 38, 40 and 41, which received calcium chlorid in addition to sodium bicarbonate, showed no such chlorotic symptoms. The chlorotic condition shown in figure 19 is much more pronounced than that due to the absence of potassium as noted in one of our previous papers<sup>19</sup> (pl. 5, fig. 2), where recovery subsequently followed.

Figure 20 shows the root systems of trees 33 and 35 and of tree 1 which received Hoagland's nutrient solution. Figure 21 shows the root systems of trees 40 and 41 and of tree 1. The root systems of trees 30-35 were very poor, and contained many dead slimy rootlets.



Fig. 20. The effect of solutions containing sodium bicarbonate without calcium on the development of the root system of orange trees. No. 1 grew in a culture receiving a complete nutrient solution; Nos. 33 and 35 grew in cultures receiving nutrient solutions containing 1000 p.p.m. sodium bicarbonate but without calcium (compare fig. 21).



Fig. 21. The effect of solutions containing sodium bicarbonate plus calcium on the development of orange tree roots. No. 1 grew in a culture receiving a complete nutrient solution; Nos. 40 and 41 grew in cultures receiving a complete nutrient solution plus 1000 p.p.m. sodium bicarbonate (compare fig. 20).



The root systems of trees 38, 40 and 41 were small compared with those grown with Hoagland's complete solution, but contained few rotting or slimy rootlets.

Several determinations were made of the  $P_H$  of the drainage waters from cans 30-35 and 36-41. It was found to range from that of the culture solution applied to faint alkalinity to phenolphthalein, that is, from  $P_H$  7.5 to  $P_H$  8.5. This indicates that bicarbonates in sand cultures may be converted to some extent into carbonates. The alkalinity present in these sand cultures no doubt greatly reduced the amount of iron available for the rootlets as described in another paper,<sup>22</sup> and also greatly reduced the available supply of phosphate and calcium. The first 500 c.c. of percolate obtained from the cultures upon adding distilled water showed no appreciable change in nitrate from the initial concentration. The phosphate found in the drainage water of series 30-35 showed no change in concentration from that of the solution applied, while that found in the drainage water of series 36-41 showed a reduction to the low average value of 4 p.p.m. Some of the original culture solution for series 36-41 was left standing a few hours and the concentration of phosphate was reduced from 105 p.p.m. to 28 p.p.m.

We cannot ignore the fact, therefore, that one of the potent effects of high OH-ion concentration is to reduce the solubility of certain other ions in the culture solution. In studying the effects of an alkaline solution upon plants, we have to take into account not alone the effect of the hydroxyl-ion concentration upon the plant, but also its effect upon the solubility of the ions in the medium which bathes the rootlets.

Samples of the various portions of trees 30-35 and trees 38, 40 and 41 were analyzed and the results are given in table 26 as percentages of dry matter and of ash.

Total nitrogen and phosphorus were greatest in the poorest trees (30-35). The total sulfur was determined only in the leaves, and by comparison with the  $SO_4$  found in the ash it appears that an appreciable percentage of the total sulfur exists in the organic form. We find that the phosphorus can be largely accounted for as  $PO_4$  in the leaves and shoots, organic combinations of phosphorus being inappreciable in amount.

An important point in the data of table 26 is the very low percentage of sodium in the ash of the leaves and shoots, and the fact



that the trunk, root and rootlets contain greater percentages of sodium than the leaves and shoots. Thus far in our studies upon absorption and distribution of elements throughout citrus trees, we have always found a lower percentage of sodium in the ash of leaves and shoots than in the other portions of the tree, and the percentage of sodium has thus far never been found to be relatively high.

All parts of the trees are relatively rich in potassium, the percentage decreasing as we pass from the leaves to the rootlets where there is an increase over that found in the root. The cells of orange trees absorb large amounts of potassium when it is available. In this respect they resemble animal cells, which may accumulate large amounts of potassium, as has been shown by Mitchell and Wilson<sup>13</sup> and others. The amounts of chlorin in the two sets of trees correspond in a way to the concentrations of chlorid furnished them. The repeated losses of older leaves may account for the low Cl content of the leaves, especially in trees 30-35.

The absence of calcium from the culture solution of series 30-35 is reflected most conspicuously in the leaves, shoots, and rootlets, indicating that the calcium of the trunk and root is relatively immobile. Lee<sup>10</sup> has reported the occurrence of mottle-leaf on citrus in the Philippine Islands which is correlated with the species of rootstock employed. It is desirable that studies be made of the calcium requirement and the calcium-transporting power of these rootstocks. At present we cannot say whether a deficiency of calcium in the leaves is due to low calcium mobility or low conductivity through the woody portions of the tree, or whether the alkaline medium which bathes the rootlets has changed the permeability of the cells or has brought about the almost complete precipitation of certain essential ions from the nutrient solution, thereby preventing the plant from obtaining an adequate supply of such ions.

In addition to chlorosis in series 30-35 there was also evidence of mottling. As has been stated, however, the leaves on trees 30-35 fell at so early a stage that it was not possible to follow their condition for any considerable length of time. No initial stages of mottle-leaf were evident in series 36-41, though growth was greatly reduced, the frequent renewal of solution, no doubt, having supplied the trees with sufficient calcium and other ions to maintain themselves. We doubt very much if mottle-leaf is the direct result of an alkaline nutrient

medium. If mottling is found to be present where the culture medium is alkaline, it is possible that it is the result of secondary causes induced by high alkalinity.

#### X. RELATIONS BETWEEN CALCIUM DEFICIENCY AND CHLOROSIS

In the course of these experiments on the effects of certain salts on the physiology of the orange tree, we have accumulated evidence of a peculiarly close relationship between calcium deficiency and chlorosis. The relation of iron deficiency to chlorosis is well known and it has been shown that a concentration of OH ions which greatly reduces the solubility of iron will induce chlorosis in plants. Several other causes of chlorosis are known so that it is evident that more than one cause may produce this effect. Our object is to call attention to the way in which a deficiency of calcium may cause chlorosis.

We have previously described<sup>18</sup> the chlorotic condition of orange leaves where the trees received a culture solution lacking calcium, but containing sodium salts in considerable quantities. It was shown that such trees lost their leaves prematurely and produced a succession of small chlorotic leaves which were likewise prematurely lost. It is not to be concluded from these observations that the absence of calcium from the culture solution was the sole cause of the chlorosis.

Since the appearance of the publication just cited we have obtained additional data from trees 60-67 grown in sand cultures. The trees were planted in May, 1921, and received a culture solution containing high NaCl and low CaCl<sub>2</sub> in the ratio of 98 NaCl: 2 CaCl<sub>2</sub>. The composition of the solution was the same as that applied to trees 6-11 in the publication just cited, except that it contained the small amount of CaCl<sub>2</sub>.

For a time trees 60-67 produced good foliage and shoots, but during the winter period of comparative dormancy all the leaves were shed from the trees and many of the shoots died. From the time this symptom appeared the trees were given a complete nutrient solution containing double the usual quantity of salts but lacking the excess NaCl formerly included. New leaves were produced the following spring, but none survived long except those on short shoots arising from the lower portion of the trunk. Many of these leaves were shed and were followed by other crops of leaves. Most of the leaves on the short shoots reached full size, but very few had the normal green color.



Some of them were entirely albescent, in some the veins were green though the tissue between them was pale green or yellow (fig. 22).

When the trees were removed at the termination of the experiment, we found that the greater part of the root system was dead. It appeared that the trees had first produced a fair root system, but that they reached a limit when the small amount of Ca at their disposal was inadequate. The terminal parts of many of the older roots died and then new roots were produced from the living region near the primary root, but many of the second roots likewise died back for



Fig. 22. Chlorotic leaves from orange trees in cultures which received a small amount of calcium in comparison with sodium.

a distance from the apical end. The failure of the trees to produce new roots after the addition of a complete nutrient solution seems to be related to the absence of green foliage. The lack of a suitable supply of carbohydrates may have been an important factor in producing this condition.

We have found chlorotic trees in cultures where the calcium supply was not limited but where faulty drainage caused the roots to die. In such cases the leaves were not shed, and the trees recovered after suitable drainage was provided. The chlorotic condition of leaves generally appears when orange roots die and decay to any considerable extent.

We found that the addition of sodium bicarbonate to the culture solution restricted the growth of roots and shoots and caused a definite



type of chlorosis where calcium was deficient. It is more than likely that the alkalinity of the culture solutions tended to precipitate certain ions, though the injury observed was not due entirely to the deficiency of nutrient ions.

## XI. INTERRELATIONS BETWEEN POTASSIUM AND CALCIUM

There is an increasing amount of evidence of an opposite relationship between the proportions of potassium and calcium in the citrus tree. Kelley and Cummins<sup>8</sup> have reported on the relationships between these ions in healthy and mottled leaves of certain species of citrus growing in the field. They showed that citrus leaves affected with "mottle-leaf" contain more K and less Ca than normal leaves of the same age. The writers<sup>18</sup> have published further evidence obtained from young orange trees grown in sand cultures under controlled conditions. The ash of trees grown in control cultures contained approximately 25 per cent K and 19 per cent Ca. The ash of trees to which calcium-deficient solutions were applied contained approximately 50 per cent K and 1 per cent Ca. Conversely, the ash of trees to which potassium-deficient solutions were applied contained approximately 30 per cent Ca and 1 per cent K. The writers have found other instances which indicate that the relations between K and Ca in the citrus tree stand in intimate connection with growth and other physiological processes.

Four series of trees in sand cultures (table 27) were grown under conditions previously described. The control series JJ (trees 105-110) received Hoagland's nutrient solution, double strength; series KK (trees 42-46) received nutrient solution (single strength) in which K was substituted for Ca; series LL (trees 111-116) received nutrient solution containing 311 p.p.m. additional K as KCl; series MM (trees 123-129) was like the series KK with the addition of 15 p.p.m. Ca as  $\text{Ca}(\text{NO}_3)_2$ .

The trees in the control series JJ made very satisfactory growth and showed no indications of mottle-leaf at any time during the course of the experiment. The leaves were dark green; the shoots and roots were well developed. The trees in series KK made very restricted growth. Their leaves showed the effects of calcium deficiency previously described, viz., premature abscission, chlorosis accompanied by small spots of dead tissue, and abnormal curling. Their roots grew



poorly and died prematurely. These conditions are reflected in the data (table 27) on the number of leaves and dry weight of trees.

The trees in series LL made good growth and were quite similar to those in the control series. Their foliage was different from that of the controls in that many of the young leaves had small yellow areas in the marginal tissue between the larger veins (fig. 23). The remainder of the leaf tissue was dark green. The yellow areas were covered subsequently with small brown papillae which were quite



Fig. 23. Orange leaves from series LL which received nutrient solution plus potassium chlorid. The marginal chlorotic spots show numerous brown papillae.

characteristic of this type of injury. As time went on the yellow areas became pale green, but they never attained the normal green color of the rest of the leaf. These affected leaves had no tendency to premature abscission. The roots of these trees compared very favorably in size and development with those of the control trees.

Trees in series MM made fairly good growth for a time, but later their leaves began to show yellow circular spots in the tissue between veins (fig. 24). These spots soon became confluent and involved much of the leaf area. Most of the leaves affected in this way fell from the trees prematurely and were followed by other crops of leaves which were smaller, were chlorotic about the margins, and bore brown

papillae on the chlorotic area (fig. 25). While the symptoms resembled those noted for leaves in series LL, they were generally indicative of more severe injury. We noted also that the injury was more severe on leaves produced during the hot summer months. Many of the depauperate leaves produced in the succeeding crops had no green tissue except near the veins and midrib (fig. 26) and therefore resemble closely the condition known as "mottle-leaf" or "frenching." The photomicrograph of a leaf section (fig. 27) shows something of the



Fig. 26. Orange leaves from series MM showing the mottled depauperate condition finally attained.

nature of the papillae on the affected leaves. The illustration shows two elevations covered with a thick dense layer. The elevations appear to have been produced by a proliferation of cells of the palisade layers. The thick superficial layer was seldom ruptured and we found no indication of parasites in the affected areas. Only a portion of the leaves on the trees in series MM were affected in the manner described; the remainder made very satisfactory growth without showing any of the symptoms noted.

Fig. 28 shows a representative tree from each of the two series LL and MM. It will be seen that there was more defoliation in the case of MM where only 15 p.p.m. calcium was supplied to the culture than in the case of LL where 159 p.p.m. calcium was supplied.







Fig. 24. Orange leaves from series MM which received nutrient solution deficient in calcium plus potassium chlorid. These leaves show an early stage of injury which resembles mottling.



Fig. 25. Orange leaves from series MM showing more advanced stage of injury. The yellow areas are covered with small brown papillae.



Fig. 29. Orange leaves from trees which received a magnesium-free nutrient solution for two years.





Fig. 27. Photomicrograph of a section of an orange leaf bearing papillae like those shown in figures 23 and 25.



Fig. 28. Representative trees from series LL and MM. The partial defoliation of the trees from series MM is apparent.



The root system of trees in series JJ and LL was large and well developed, but in MM the roots made restricted growth and contained many rotten rootlets.

The percentage of K (table 27.) was generally higher in the ash of leaves and rootlets (except when roots died) than in other parts of the trees. The ash of trunks and roots had considerably smaller percentages of K than other parts of the trees. The leaves and shoots of the trees in series KK were notably high in their K content, although the trees received the same concentration of K in the culture solution as trees in series LL and MM. The K content of these trees shows an inverse relationship to Ca content similar to that which has been discussed previously. The parts of the trees which showed the most serious effects were those which had the highest per cent of K in their ash.

The Ca content of the trees corresponded in a qualitative way with the Ca content of the culture solutions, that is, the trees in series KK which received no Ca contained the least calcium, and trees in series JJ which received a solution containing 318 p.p.m. Ca contained most. The trunks and roots in most cases contained more Ca than other parts of the trees.

The variations in the per cent of Mg in the ash were small and bore no apparent relation to the condition of the trees.

The Cl content of the ash was, in no case, high enough to be responsible for any of the injurious effects observed. In fact, the trees in series KK and MM which showed injury contained negligible amounts of Cl.

Analyses of the affected leaves from series MM showed minor differences in the content of certain ash constituents. Their composition was:

	Per cent of ash	Na	K	Ca	Mg	Cl	SO <sub>4</sub>	PO <sub>4</sub>
Affected .....	13.43	9.77	45.27	4.00	1.66	.32	3.86	3.54
Healthy .....	13.58	9.30	41.38	6.18	1.80	.33	3.79	3.35

The amounts of the ions are expressed as percentages of the ash. The leaves were selected as closely as possible for uniform age. The differences in composition, while small, agree with those found by Kelley and Cummins<sup>8</sup> in the case of normal and mottled leaves.



TABLE 28  
GROWTH AND COMPOSITION OF VALENCIA ORANGE TREES IN RELATION TO MAGNESIUM

Series	NN	OO
Culture solutions contained.....	14 p.p.m. Na 108 p.p.m. Mg 210 p.p.m. PO <sub>4</sub>	110 p.p.m. Na - 0 p.p.m. Mg 105 p.p.m. PO <sub>4</sub>
Osmotic pressure of solution.....	1.452 atmospheres	.965 atmospheres
Number of leaves per tree.....	1,351	1,379
Dry weight per tree.....	1,417	1,486
Units of water transpired per unit of dry matter.....	319	317
Per cent in the ash:	NaKCaMgSO <sub>4</sub> PO <sub>4</sub>	NaKCaMgSO <sub>4</sub> PO <sub>4</sub>
Leaves.....	4.5818.2320.881.854.394.70	5.9820.9419.57.574.073.78
Shoots.....	3.2914.8922.952.404.867.77	4.0214.7024.66.622.815.02
Trunks.....	3.4213.9722.701.683.756.82	5.1615.3522.251.156.57
Roots.....	3.7115.5917.732.352.688.15	6.6812.4317.221.176.50
Rootlets (silica-free basis).....	7.0928.337.803.7112.5212.05	12.1922.096.10.8012.297.51

The results of this experiment bring out some facts concerning the relations between K and Ca which seem worthy of notice. The marked injury shown by trees in series KK was so similar to what has been observed in other cases that it can be ascribed principally to Ca starvation. The yellow spots on the leaves, the premature abscission, and the gelatinous dead roots were characteristic of Ca starvation. The effects of Ca deficiency were so great as to make it difficult to distinguish any other effects.

In series LL the injurious effects were insignificant or at least transient; on the contrary, the injurious effects noted in series MM were obvious and became more severe as time went on. It would appear that, in the latter case, the amount of Ca furnished the trees was sufficient to prevent the most acute results of Ca starvation though it did not prevent the trees from taking up large amounts of K. In the case of series LL, the larger concentration of Ca furnished, changed the proportion of K and Ca in the ash, and allowed the trees to make satisfactory growth.

Attention may be called to the fact that, in a large series of experiments upon the effects of various salts upon orange trees, the writers have not hitherto obtained results which resembled as closely the condition known as "mottle-leaf" as those here reported. It appears to us that the development of mottle-leaf is related to certain conditions in which the amount of K present greatly exceeds that of Ca. This is not to imply that we think there is a particular proportion between K and Ca which causes this condition; on the contrary, there may be a rather wide range of conditions which produce a similar result. The concentration of monovalent ions may be in excess of that of Ca without producing effects on the trees such as here described. For example, another series which received a culture solution containing concentrations in parts per million as follows, K 185, Na 400, Ca 159, and Cl 617, showed none of the symptoms of mottling found in trees of series LL nor of mottling and abscission found in trees MM.

The H-ion concentration of the sap of healthy mature leaves from series LL and MM showed no significant difference. The former had a  $P_H$  of 5.82 and the latter 5.92, as determined electrometrically. Their titration curves were practically identical.

## XII. EFFECTS OF MAGNESIUM DEFICIENCY ON YOUNG ORANGE TREES

Six trees were planted in the sand cultures April 5, 1921, and removed January 5, 1923. The care of the cultures was like that described in the preceding paragraphs. The composition of the magnesium-free culture solution in parts per million of ions was

Na	K	Ca	Mg	Cl	NO <sub>3</sub>	SO <sub>4</sub>	PO <sub>4</sub>	Fe	Mn
110	185	159	0	10	718	214	105	1	0.1

giving a total concentration of 1502.1 parts per million. The control series NN grown simultaneously received double-strength nutrient solution.

The trees in the magnesium-free series 00 grew well and attained a development at the end of the period mentioned equal in all respects to those in the control series NN (table 28). The only significant difference was a peculiar chlorotic stripe on the older leaves and this character did not appear until the experiment had been in progress about a year. The nature and development of this chlorotic condition are shown in fig. 29. Between the larger veins in the initial stages, there was a series of yellow areas situated a short distance on either side of the midrib of the leaf. As these yellow areas enlarge they coalesced to form a well-defined stripe in the middle of the leaf and gradually covered the veins and midrib. The bleaching was most pronounced at the base of the leaf. In course of time the chlorotic area extended toward the margin of the leaf as shown at the right of fig. 29, and eventually many leaves were entirely chlorotic. We noticed no premature abscission of these chlorotic leaves. Only part of the leaves on any tree showed this condition and they were usually in the lower, interior part of the tree.

The composition of various parts of the trees (table 28) shows few significant differences when compared with that of the control trees. The principal difference was in the Mg content, as one might expect. The lowest content of Mg was found in the ash of leaves, shoots, and rootlets. We have previously shown<sup>23</sup> that the Mg of the trunk and root of an orange tree is somewhat less soluble than that of other parts of the tree, and it is possible that the original content of Mg in these trees had not been entirely reduced by transfer to the more actively growing portions of the tree.

Attention may be called to the significance of this fact in the nutrition of trees. Although the tree when planted contained about 2 per cent of Mg in its ash, this small amount was sufficient to maintain good growth and no untoward effects of any kind were observed during the first year. Somewhat similar results were noted when orange trees were deprived of potassium, but, on the contrary, injury was soon apparent when trees were deprived of calcium.<sup>19</sup> Trees deprived of Mg showed no striking changes in their content of other ions, although there was some reduction in the amount of  $\text{PO}_4$  in the ash of the rootlets.

In view of these results we may assume that a relatively small amount of magnesium is adequate for satisfactory growth and chlorophyll production. This assumption is supported by the fact that good growth is obtained when the trees are given a nutrient solution containing Mg in a concentration of 54 parts per million.

## SUMMARY

The data presented in the preceding pages give some of the results obtained from several series of experiments with seedlings and young trees. They are designed to give some information on the phenomena of absorption of ions and the consequent effects upon growth. Walnut seedlings absorbed sulphate in increased amounts when the concentration of that ion in the culture solution was increased, although the absorption was by no means parallel to the concentration. Seedlings supplied with a culture solution containing 1200 p.p.m. sulphate made very good growth, but, in higher concentrations growth was restricted and leaves were killed at the margins.

Concentrations of nitrate as high as 1600 p.p.m., furnished as sodium nitrate, had no detrimental effects on the growth of walnut seedlings, and higher concentrations only had a retarding influence upon the growth of tops. An initial concentration of 2700 p.p.m. nitrate produced injury to the margins of walnut leaflets similar to that produced by a concentration of 1500 p.p.m. chlorin as NaCl. A comparison of the growth attained in equi-molecular concentrations of sodium nitrate and sodium sulfate indicated that the latter is the less favorable.

Walnut and orange trees absorb chlorin readily and their growth is characteristically affected by amounts which would be harmless in the case of certain other anions. The roots of these seedlings usually contain more chlorin than the tops, though the leaves of orange trees may contain as high as 22 per cent chlorin in their ash. Between  $P_H$  6.0 and 9.0 the reaction of the culture solution seemed to have little influence upon the amount of chlorin in the ash of walnut seedlings. Rough-lemon seedlings grown in a similar range of  $P_H$  values had slightly more chlorin in the ash of tops at  $P_H$  9.0 than at lower values. The smallest percentage of chlorin in the ash of their roots was found in plants grown at  $P_H$  7.0, and the amounts in roots grown at  $P_H$  6.0, 8.0, and 9.0 were not significantly different.

Young orange trees grown in soil cultures were seriously injured by the application of irrigation water containing 880 p.p.m. chlorin (1500 p.p.m. NaCl). The leaves turned yellow and showed dead margins which are typical of salt injury. The chlorin content of the injured leaves was very high, and there was no evidence that chlorin migrates from the leaves prior to abscission, although it was soluble in water.

Soils containing harmful amounts of sodium chlorid were leached under controlled conditions and their salt content was reduced to a point which permitted the trees to make satisfactory growth, although it was necessary to add nutrient salts.

A concentration of 1000 p.p.m. of sodium bicarbonate restricts the growth of walnut roots and often prevents completely the development of the epicotyls. The application to young orange trees of culture solution deficient in calcium and containing sodium bicarbonate, retarded the growth of the tops and eventually killed the roots. The orange leaves first showed abnormal curling and translucent spots, followed by premature abscission. Many of the leaves developed chlorosis.

The injurious effects of high concentrations of potassium ions were not evident until considerable time had passed. Walnut seedlings in culture solutions containing 1700 p.p.m. potassium made good growth for a time, but eventually the foliage turned yellow and the roots ceased to grow. Young orange trees in sand cultures which received solutions containing 500 p.p.m. potassium grew well for the first year, but eventually their leaves developed characteristic yellow spots on which brown papillae appeared. Many of the depauperate



leaves subsequently produced resembled mottled leaves. These untoward symptoms were more severe in a series of cultures where the amount of calcium furnished was very small. The leaves and rootlets showed most severe injury from this cause and their ash was richer in potassium.

The data show that sodium is not severely toxic unless the concentration is fairly high. When a sodium salt was added to a complete nutrient solution the trees were not severely injured except in cases where concentration as such became a factor. The growth of the epicotyl of walnut seedlings was retarded by sodium salts more than the growth of roots; however, the roots were characteristically injured later.

Walnut seedlings made thrifty growth in solutions of calcium chlorid, and very restricted growth in equi-molecular solutions of sodium chlorid. Strong concentrations of chlorin as calcium chlorid were somewhat less toxic to rough-lemon seedlings than equivalent concentrations as sodium chlorid. When calcium was furnished in sub-minimal amounts, the roots of young orange trees died and the foliage was chlorotic. The ash of the trunk and the main root of the orange trees was richer in calcium than other parts of the tree, though in certain cases the older leaves were very rich in calcium. Additional evidence may be gathered from these experiments to support the idea that the absorption of calcium and potassium presents a sort of antithesis in the function of the citrus tree. If the amount of one element is high in the ash, the amount of the other will be low. The roots of walnut seedlings are extremely sensitive and are quickly injured in calcium-free solutions.

Culture solutions whose reaction was on the alkaline side of the neutral point were not detrimental to the growth of rough-lemon seedlings; in fact, they made more growth at  $P_H$  8.0 than at  $P_H$  6.0 or lower. Citrus and walnut seedlings grown at  $P_H$  values of 7.0, or higher, contained as much chlorin in the ash of their tops as those grown at lower  $P_H$  values. The  $P_H$  values of the sap expressed from walnut seedlings were quite uniform regardless of the reaction of the solution in which they were grown.

A concentration of 1500 p.p.m. sodium sulfate caused no apparent injury to walnut seedlings, but at a concentration of 3000 to 6000 p.p.m. sodium sulfate the growth of the seedlings was progressively

retarded. Increasing concentrations of sodium nitrate had somewhat the same effect.

It is very difficult to anticipate the effects of a given concentration of an ion in the culture solution upon the amount of that ion found in the plant, although, in a general way the per cent found in the plant reflects the concentration in the culture solution. The data show, however, that the variations are by no means proportional.

On account of its mobility in the plant we might expect to find chlorin rather uniformly distributed, but we find that it has a tendency to accumulate in the roots of orange trees and in the epicotyls of walnut seedlings.

The effect of a very low concentration of magnesium on orange trees was mainly evident in a peculiar type of chlorosis in which only the tissue along the midrib of the leaf was involved. In spite of a low content of magnesium in the ash of the trees, there was no evidence of any profound physiological disturbance.

The effects of sodium chlorid on trees growing in soils under controlled conditions throw some light upon certain conditions often seen in the field. The restricted growth of roots and shoots, and the premature abscission of leaves agree with the analyses which show an increased chlorin content of those members. The conditions for growth in saline soil were greatly improved as a result of leaching the cultures while the trees were growing in them.

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FACTORS INFLUENCING THE RATE OF  
GERMINATION OF THE SEED OF  
ASPARAGUS OFFICINALIS

BY

H. A. BORTHWICK

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I. INTRODUCTION

Asparagus seed under average field conditions germinates slowly. In the Delta Region of the Sacramento River, which is the largest asparagus growing section in the United States, it is usually from two to six weeks before the seedling appears above the surface. This variation is due chiefly to differences in the temperature and moisture of the soil and in the depth of planting. Seed planted early is particularly slow in coming through the soil on account of the low

soil temperatures which prevail during the early part of the season. Even at these low temperatures, however, weeds may soon cover the ground and obscure the rows, making cultivation difficult. It has, in fact, been the custom in some asparagus growing sections to plant such quick-growing plants as radishes in the rows of asparagus so that the grower may see the rows before the asparagus appears and cultivate between them to destroy weeds.

No record has been found of any carefully controlled experiments to determine the influence of physical and chemical treatments upon the germination of asparagus seed. Hexamer<sup>1</sup> reports that "soaking the seed in lukewarm water for twenty-four hours before planting will hasten germination." Among growers there seems to be a diversity of opinion as to the value of soaking asparagus seed. Soaking asparagus seed before planting is practiced by a number of growers who assert that the plants come up a week or more ahead of those from unsoaked seed. Other growers maintain that they can see no difference in the rate of germination of soaked and unsoaked seed. Treatments other than soaking in water seem not to have been tried at all or at least not successfully, as no records have been found.

It is the purpose of this investigation to ascertain whether the period between the planting of asparagus seed and the appearance of seedlings above ground may be shortened by soaking in water. The temperature of the water, and the time of soaking are the factors involved.

Work is now in progress having to do with the influence of various physical and chemical treatments upon the germination of asparagus seed.

## II. LITERATURE

Numerous records are found in the literature having to do with the effect of soaking in water on germination of the seeds of various plants. A survey of such records shows that considerable variation exists in the results obtained. First, the seeds of different plants soaked in water under identical conditions may give entirely different germination results. Kidd and West<sup>4</sup> found that broad beans gave a better germination as a result of soaking, whereas a similar treatment decreased the germination of dwarf beans. Coupin<sup>6</sup> shows that many kinds of seeds are killed by soaking two weeks or even less but



that asparagus seed may survive 20 weeks' soaking. Second, seeds of the same kind of plant give markedly different germination results when the conditions under which they are soaked are varied. Coupin<sup>6</sup> finds that asparagus seed remains alive only 75 days in water which is changed frequently, but 145 days in unchanged water. On the other hand, he finds that beets will remain alive five times as long if the water is changed every day as they will if left in the same water the entire time.

Wollny<sup>3</sup> and Kraus<sup>2</sup> find that the amount of water in which certain seeds are soaked has much to do with their rate of germination.

These few brief references to the literature make obvious the fact that it is unsafe to predict the effect of soaking treatments on any kind of seed from results of similar treatments obtained with some other kind of seed. Moreover, the importance of controlling and describing the conditions under which the soaking treatments were carried out is emphasized.

### III. METHODS

*Source of Seed.*—The seed used in these experiments was obtained from the California Packing Corporation. It was grown on their ranch on Ryer Island in the Lower Sacramento Valley. Most of the experiments were made with two year old seed although some one year old seed was also used. Control experiments, however, showed no difference in germination of one and two year old seed.

Seeds soaked in water, in most cases, were soaked in uncorked bottles containing 100 cc. of water. The bottles were of such diameter that there were never more than two layers of seeds on the bottom. The seeds were covered by approximately five centimeters of water. They were placed in ovens, the temperatures of which did not fluctuate more than one degree centigrade. The duration of the soaking was accurately timed. This method of soaking is not in accord with the method adopted by Wollny,<sup>3</sup> and certain other workers who soaked their seed in as small an amount of water as possible in order to prevent excessive exosmosis of soluble food reserves. Wollny records that a reduction in germination and vigor of many of the seeds with which he worked, followed soaking in excessive amounts of water. I could find no such effect in the case of asparagus seed. For



this reason no attempt was made to keep the volume of water constantly small in the various experiments. It is of interest in this connection to note that Coupin<sup>6</sup> found that asparagus was able to withstand more than twice as much soaking as any other seed of twenty-three kinds he studied, provided no fresh water was added throughout the experiment.

*Germination Tests.*—Germination tests were made in the laboratory and in the field. In the laboratory tests, samples of 100 seeds each were germinated on cloth germinators made of Canton flannel strips 8 inches by 32 inches which by folding twice gave an 8 × 8 inch germinator four layers thick. The seeds had two layers above and two below. These germinators were placed in a constant temperature chamber in piles of not more than three each. Where the temperature of the germination chamber was other than 30° C., a note of it is made in the text. The germinators were kept moist by sprinkling once or twice a day. In the field tests, samples of 100 or 200 seeds were used. After treatment they were planted in rows about 2 inches deep.

The period of germination was reckoned from the time the seed was planted in the field or placed in the germinators until the first evidence of germination was visible. This in the laboratory tests was at the rupture of the seed coat—in the field at the emergence of the growing tip of the seedling. The field germination periods were, therefore, longer than corresponding laboratory germination periods because the shoots had to elongate at least two inches before they could be seen.

*Water Intake Determinations.*—Before water intake determinations were made, the seeds were carefully examined and all cracked and shriveled seeds discarded. The good seeds were then weighed in the air-dry condition. After various periods of soaking, the seeds were removed from the water and dried quickly with filter paper until the seed coats ceased to glisten. They were then reweighed and returned to the water as quickly as possible. Where determinations were made at close intervals of from one to three hours, the time recorded is the time the seeds were actually in the water and does not include the time during which the weighings were being made. When the determinations were made at greater intervals, however, the time is figured from the time the seeds were first immersed.

IV. EXPERIMENTAL DATA AND DISCUSSION

Asparagus seeds placed under suitable germinating conditions will germinate, whether taken directly from the mature berry, or from storage after one or two years. In fact, it is possible to get germinations of 90 per cent or more from untreated seed in one week in the laboratory, provided the temperature remains around 30° C. These facts indicate that we are not concerned here with a seed having a definite period of dormancy. If germinations of 90 per cent in one week could be obtained in the field, it would be unnecessary to hasten germination. At the time asparagus nurseries are usually planted, however, soil temperatures range from 20° C. down to about 10° C. At these temperatures asparagus seed germinates very slowly, as shown in table 1.

*Effect of Temperature upon Germination.*—The following table shows the effect of temperature upon the rate of germination of untreated asparagus seed.

TABLE 1  
RATE OF GERMINATION OF UNTREATED ASPARAGUS SEED, GERMINATED AT DIFFERENT TEMPERATURES IN THE LABORATORY

Temperature germinating chamber (° C.)	Percentage germination after:							
	3 days	4 days	5 days	6 days	8 days	10 days	12 days	17 days
10	0	0	0	0	0	0	0	0
20	0	0	2	4	11	14	.....	27
25	0	25	65	84	98	98	98	98
30	0	50	74	83	91	95	96	97
35	0	5	.....	16	31	55	67	.....
40	0	0	0	0	0	0	0	0

It will be seen from this table that the optimum temperature of germination for untreated asparagus seed is from 25° to 30° C. Germination is very slow at 20° C., and rather slow again at 35° C.

*Rate of Water Intake of Seeds Soaked at Different Temperatures.*—It was thought that determinations of the rate of water intake by asparagus seed at various temperatures might shed some light on the

cause of slow germination, so determinations were made at several temperatures between 10° and 40° C. The results are shown in table 2 and figure 1.

TABLE 2  
RATE OF WATER INTAKE BY ASPARAGUS SEEDS SOAKED AT DIFFERENT TEMPERATURES

The percentage of increase in weight is based on the original air dry weight.

Temperature soaked (° C.)	Percentage increase in weight due to soaking after:					
	4 hours	10 hours	15 hours	16 hours	22 hours	24 hours
10			11.7			14.4
18	6.1	14.4		19.6	24.0	
22	6.1	15.8		21.2	27.0	
30	15.9	27.5		33.0	36.0	
40	27.6	36.5		38.4	38.9	

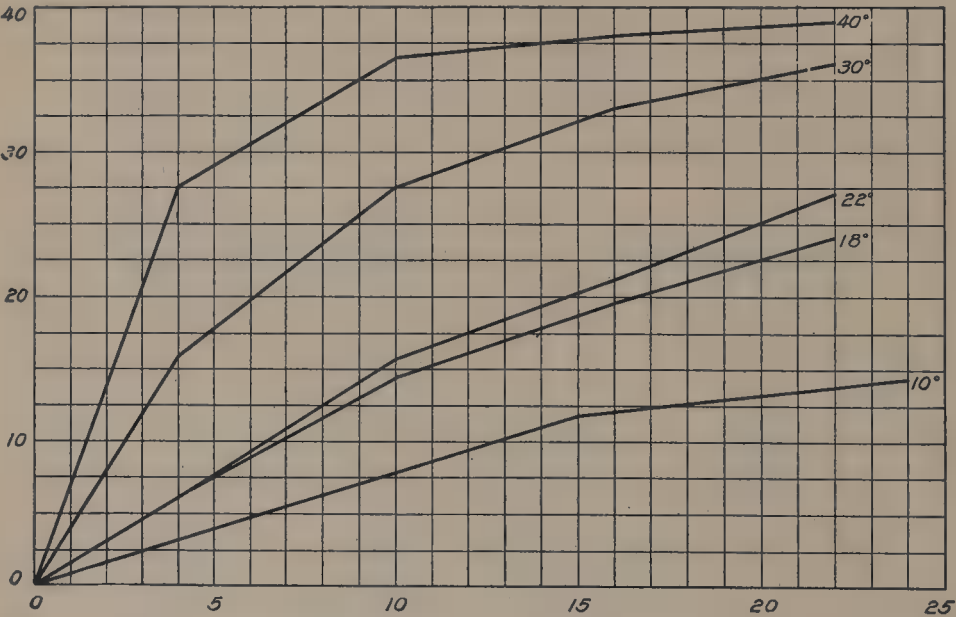


Fig. 1.—Rate of water intake by asparagus seed soaked in water at temperatures of 18° C and 30° C.

At a later date determinations of the rate of water intake were again made at temperatures of 18° and 30° C., and weighings were made for 164 hours. These results appear in table 3 and figure 2.

The results presented in table 3 and figure 2 show that seeds immersed in water at 30° C. take up very nearly their maximum amount of water about 30 hours earlier than seeds soaked at 18° C.

*Germination after Soaking in Water.*—Germination tests, in which seeds were soaked in water at different temperatures and for different lengths of time, were carried out both in the laboratory and in the field. These experiments were repeated a number of times with very uniform results.

TABLE 3

RATE OF WATER INTAKE BY ASPARAGUS SEEDS SOAKED AT TEMPERATURES OF 18° C. AND 30° C.

Temperature soaked (° C.)	Percentage increase in weight following periods of soaking of:											
	3 hours	9 hours	24 hours	33 hours	48 hours	54 hours	69 hours	93 hours	117 hours	142 hours	153 hours	164 hours
18	5.4	13.2	28.7	34.5	40.2	41.1	42.6	43.2	43.3	.....	43.6	43.5
30	9.6	22.4	38.7	41.6	42.4	42.7	42.6	42.8	42.9	43.4	43.2	43.2

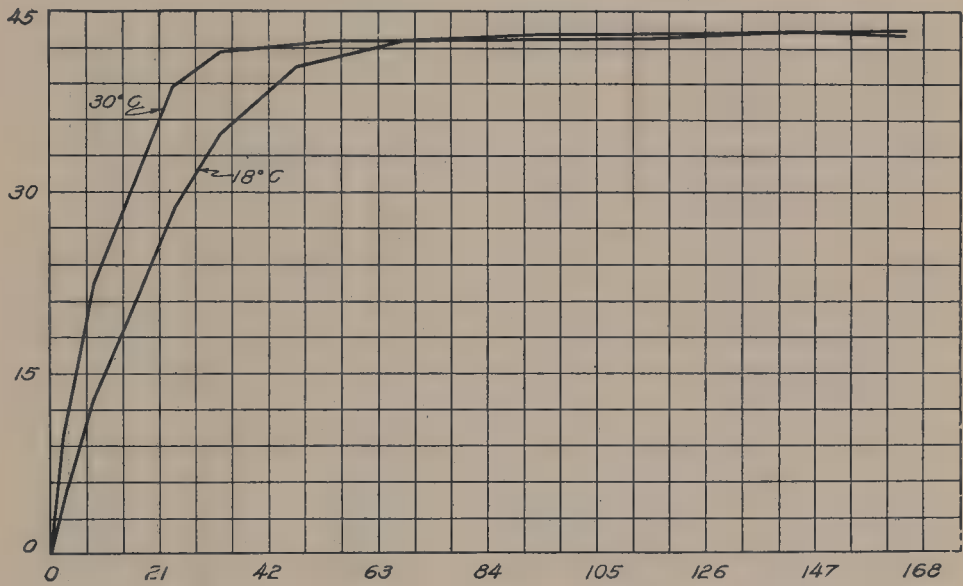


Fig. 2.—Rate of water intake by asparagus seed soaked in water at various temperatures.

*Laboratory Tests.*—In tables 4*a* and 4*b* the results are given of a series of treatments in which seeds were soaked in water for various lengths of time at temperatures ranging from 22° C. to 50° C. Daily germination percentages are shown for thirty-six sets. In Table 5 are presented data of another series similar to those of table 4*a* and 4*b*. Germination was carried out at 30° C. instead of at the somewhat lower and more variable room temperature used with the series of

TABLE 4a

EFFECT ON GERMINATION OF SOAKING ASPARAGUS SEEDS IN WATER AT VARIOUS TEMPERATURES AND FOR DIFFERENT PERIODS OF TIME. GERMINATED AT ROOM TEMPERATURE IN THE LABORATORY.

Temperature soaked	Hours soaked	Per cent germination after:								
		2 days	3 days	4 days	5 days	7 days	8 days	9 days	10 days	11 days
22° C.	6	3	39	65	77	85	86	88	88	88
	14	2	13	56	66	89	90	93	93	93
	20	0	11	50	86	97	98	98	98	98
	38	12	51	75	81	88	89	90	90	92
	62	37	66	82	88	96	96	97	97	97
	86	41	64	86	88	92	93	94	94	94
	110	52	69	85	89	96	96	96	96	96
30° C.	6	0	12	54	74	93	94	95	95	95
	14	0	30	69	81	91	93	93	93	93
	20	0	50	84	92	94	96	96	96	98
	38	41	62	85	93	96	97	98	98	98
	62	45	67	78	89	94	95	95	95	95
	86	45	73	88	94	98	98	98	98	98
	110	64	86	93	95	96	97	97	97	98
38° C.	6	0	21	56	83	92	95	96	96	96
	14	3	41	69	83	89	91	92	93	93
	20	4	36	71	83	87	90	90	90	90
	38	24	74	84	92	95	95	95	95	95
	62	41	73	82	90	97	97	99	99	99
	86	39	69	82	82	94	95	95	95	95
	110	38	71	81	85	89	89	89	89	89
45° C.	6	1	13	57	71	88	90	93	93	93
	14	1	18	60	73	87	88	89	89	91
	20	0	19	63	79	91	93	94	94	94
	38	0	2	36	68	88	90	93	94	94
	62	0	0	3	12	76	85	88	90	92
	86	0	0	0	0	18	39	58	74	81
	110	0	0	0	0	0	0	0	2	3
50° C.	6	0	12	55	78	85	87	87	89	89
	14	0	14	65	87	95	95	95	95	95
	20	0	2	22	58	92	94	95	95	95
	38	0	0	3	18	60	73	82	86	86
	62	0	0	0	0	5	10	24	35	44
	86	0	0	0	0	0	0	0	0	0
	110	0	0	0	0	0	0	0	0	0
Unsoaked control.....		0	1.5	31	60	79	85	89	89	95

The control is the average of the results from two unsoaked cultures.



TABLE 4b

EFFECT ON GERMINATION OF SOAKING ASPARAGUS SEEDS IN WATER AT VARIOUS TEMPERATURES AND FOR DIFFERENT PERIODS OF TIME. GERMINATED AT ROOM TEMPERATURE IN THE LABORATORY.

Hours soaked	Tem-perature soaked (° C.)	Per cent germination after:								
		2 days	3 days	4 days	5 days	7 days	8 days	9 days	10 days	11 days
6	22	3	39	65	77	85	86	88	88	88
	30	0	12	54	74	93	94	95	95	95
	38	0	21	56	83	92	95	96	96	96
	45	1	13	57	71	88	90	93	93	93
	50	0	12	55	78	85	87	87	89	89
14	22	2	13	56	66	89	90	93	93	93
	30	0	30	69	81	91	93	93	93	93
	38	3	41	69	83	89	91	92	93	93
	45	1	18	60	73	87	88	89	89	91
	50	0	14	65	87	95	95	95	95	95
20	22	0	11	50	86	97	98	98	98	98
	30	0	50	84	92	94	96	96	96	98
	38	4	36	71	83	87	90	90	90	90
	45	0	19	63	79	91	93	94	94	94
	50	0	2	22	58	92	94	95	95	95
38	22	12	51	75	81	88	89	90	90	92
	30	41	62	85	93	96	97	98	98	98
	38	24	74	84	92	95	95	95	95	95
	45	0	2	36	68	88	90	93	94	94
	50	0	0	3	18	60	73	82	86	86
62	22	37	66	82	88	96	96	97	97	97
	30	45	67	78	89	94	95	95	95	95
	38	41	73	82	90	97	97	99	99	99
	45	0	0	3	12	76	85	88	90	92
	50	0	0	0	0	5	10	24	35	44
86	22	41	64	86	88	92	93	94	94	94
	30	45	73	88	94	98	98	98	98	98
	38	39	69	82	82	94	95	95	95	95
	45	0	0	0	0	18	39	58	74	81
	50	0	0	0	0	0	0	0	0	0
110	22	52	69	85	89	96	96	96	96	96
	30	64	86	93	95	96	97	97	97	98
	38	38	71	81	85	89	89	89	89	89
	45	0	0	0	0	0	0	0	2	3
	50	0	0	0	0	0	0	0	0	0
Unsoaked control.....		0	1.5	31	60	79	85	89	89	95

The control is the average of the results from two unsoaked cultures.

TABLE 5

EFFECT ON GERMINATION OF SOAKING ASPARAGUS SEEDS IN WATER AT VARIOUS TEMPERATURES AND FOR DIFFERENT PERIODS OF TIME. GERMINATED AT 30° C. IN THE LABORATORY.

Temperature soaked	Hours soaked	Per cent germination after:						
		2 days	3 days	4 days	5 days	6 days	7 days	8 days
20° C.	12	0	13	40	63	72	.....	81
	24	2	35	56	64	70	.....	76
	48	7	38	66	78	86	.....	87
	72	12	48	66	81	84	.....	91
	96	34	60	81	88	93	.....	96
	120	39	69	88	94	95	.....	97
	144	24	62	82	89	92	.....	95
25° C.	12	1.5	23.5	71	80.5	90.5	94	94.5
	24	13.5	55	84.5	90	93	95	95.5
	48	32	66	82	87	95.5	93.5	93.5
	96	58.5	77.5	91.5	92.5	97	98	98
	216	48	71.5	94.5	94.5	96	97	97
30° C.	12	6	23	71.5	84.5	93	95.5	96
	24	16	44	77.5	80	90	92	92
	48	44.5	68.5	86	90.5	95	95.5	96
	96	61.5	75.5	92.5	97.5	99.5	100	100
	216	33	50	81	91	91.5	96.5	96.5
35° C.	12	5	37	71	85.5	91.5	94.5	95
	24	18.5	56	79.5	86	92	97.5	97.5
	48	42	71.5	88.5	93	95	97	97.5
	96	36	66.5	85.5	93	94.5	96.5	97
	216	48.5	75	83.5	94	92	94.5	95
40° C.	12	3	35	69	86	90	91.5	95.5
	24	11	50	75	89	93	96	96
	48	22.5	60	74.5	86.5	89.5	95	96
	96	20	51.5	80	86	89.5	92.5	94.5
	216	3.5	34.5	67.5	77	85	86.5	90
50° C.	12	0	6.5	25.5	37.5	40	46.5	50
	24	0	0	16.5	32	36	39.5	40.5
	48	0	0	0	0	1.5	6.5	14.5
	96	0	0	0	0	0	0	0
	216	0	0	0	0	0	0	0
Un-soaked control.....		0.2	7.4	53.6	74.2	83.8	90.2	92.8

tables 4a and 4b. For this reason and because of minor differences in temperature-time combinations used in this series, these data can not be averaged with those of table 4a.

The date on which the seeds were placed in the germinator and not the date when soaking was begun is used as the reference point for recording germinations. Germination is impossible for most seeds while immersed in water because of an insufficient supply of oxygen and a possible excess of carbon dioxide. This was shown in an experiment with asparagus seeds in which they were soaked under several centimeters of water through which air was slowly bubbled. In one week nearly all of these seeds had germinated vigorously, whereas a control soaked in unaerated water showed no germination whatever. In fact, asparagus seeds immersed in unaerated water as long as two months have failed to germinate. The fact that germination is inhibited during a period of immersion in water has been pointed out by Kidd and West.<sup>5</sup>

Table 4a is arranged in five main divisions, each of which includes all cultures soaked at the same temperature. To make comparisons of individual cultures easier, the same results are rearranged in table 4b so that cultures soaked the same number of hours are grouped together.

The data presented in this table are the average of two identical series of cultures in all cases except cultures soaked at 20° C. and the control. The data for 20° cultures are based on single cultures while the data for the control represent the average of five unsoaked cultures.

*Field Tests—Series No. 1.*—When seeds were being soaked for the tests shown in table 4a, 200 seeds were used in each set. Half of these were germinated in the laboratory and the other half in the field. The treatment received by corresponding cultures to be tested in the laboratory and in the field was therefore exactly the same up to the time they were planted. Seed was planted at a depth of approximately three inches. This series was planted July 16, 1923, a time when soil temperatures were very high and when growth was exceedingly rapid. Asparagus nurseries are usually planted between the middle of March and the middle of May or a little later. The tests reported in table 6 were made considerably later than asparagus is ordinarily planted, but it is believed that the results are indicative of what may be expected in late planted nurseries.

TABLE 6

EFFECT OF SOAKING IN WATER AT VARIOUS TEMPERATURES FOR VARYING LENGTHS OF TIME ON GERMINATION OF ASPARAGUS SEED IN THE FIELD. PLANTED JULY 16, 1923.

Temperature soaked	Hours soaked	Per cent sprouts above ground after seeds were planted:				
		12 days	14 days	16 days	21 days	28 days
22° C.	6	1	11	15	44	45
	14	2	12	26	46	63
	20	0	3	16	39	40
	38	3	17	26	51	53
	62	8	24	31	59	54
	86	11	23	27	55	60
	110	7	14	17	39	49
30° C.	6	0	5	13	39	.....
	14	9	15	25	57	61
	20	3	11	18	52	50
	38	9	11	24	49	55
	62	14	18	38	68	79
	86	19	39	52	67	76
	110	20	30	36	57	48
38° C.	6	0	8	23	66	59
	14	4	11	29	55	54
	20	6	18	30	60	57
	38	6	16	28	50	.....
	62	13	30	35	68	53
	86	8	15	27	49	49
	110	2	24	23	65	54
45° C.	6	2	5	22	54	63
	14	8	12	16	48	54
	20	1	8	18	66	64
	38	5	16	31	54	71
	62	0	1	15	54	77
	86	0	0	0	13	35
	110	0	0	0	0	4
Unsoaked control.....	.....	0	1.75	10.5	41.75	55.75

The control is the average of four unsoaked cultures.

Table 6 shows the following facts:

1. Sixteen days after planting, all cultures shown in the table except those soaked at 45° C. for 86 or more hours have a considerably higher percentage of seedlings above ground than the unsoaked control.

2. There is no appreciable difference in final germination obtained from soaked and unsoaked seeds.

*Field Tests—Series No. 2.*—Another series, treated as indicated in table 7, was planted on March 4, 1924. The depth of planting of this series was between two and two and a half inches. The soil at the time of planting was in excellent physical condition. The seeds were placed in direct contact with finely pulverized moist soil and were immediately covered before they had any opportunity to dry. The temperature of the soil at a depth of two inches, at the time of planting and until the seeds began to come up, ranged from 10° to 23° C. There were relatively few hours when the temperature was above 20° C. during the first two weeks after the seeds were planted.

This planting which was made at a date somewhat earlier than that at which asparagus nurseries are usually planted, and than the one from which table 6 is taken, represents the opposite extremes of planting season.

In table 7 as in table 6, it is seen that many of the soaked cultures begin germination much sooner than the unsoaked cultures. It is also apparent that the final percentage germination is not appreciably changed by the treatment.

The percentages of germination on the fifty-first day after planting were, in a few cases, lower than those of the forty-seventh day. This was due to the destruction of plants by insects or other causes.

A comparison of the results shown in tables 6 and 7 with those of tables 4a and 5 shows that, in the field, there are factors operating which tend to obscure the effects of the seed treatments. For example, a culture soaked 20 hours at 22° C. (table 4a) gave 98 per cent germination eight days after it was placed on the germinator in the laboratory. This result indicates that the seeds were not harmed in any way by the treatment. In fact, this culture, in the laboratory, germinated much more quickly than the controls. Yet the duplicate lot of seeds which was soaked in the same water with these just described, produced only 40 per cent germination in the field (table 6), or a 15 per cent poorer germination than the controls.

The factors which make for lack of uniformity of germination results in the field are probably differences chiefly in depth of planting and in soil moisture. Since the appearance of primary shoots above ground was taken as the first evidence of germination, an increase of an inch in depth of planting, for example, has a marked influence on the results for at least two reasons. First, the shoot has an extra inch of growth to make before it appears above ground and,



second, the seed is in a cooler layer of soil where growth is slower. Other factors, such as insect attacks, and mechanical injuries during cultivation and counting, have some influence on the results.

*Effect of Planting in Cold Soil after Soaking in Warm Water.*—It is frequently said that soaking seed in warm water before planting in cold soil is detrimental. The results of field tests (table 7), however,

TABLE 7

EFFECT OF SOAKING IN WATER AT VARIOUS TEMPERATURES FOR VARIOUS LENGTHS OF TIME ON THE GERMINATION OF ASPARAGUS SEED IN THE FIELD. PLANTED MARCH 4, 1924.

Temperature soaked	Hours soaked	Percentage sprouts above ground after seeds were planted:							
		30 days	33 days	35 days	37 days	40 days	43 days	47 days	51 days
25° C.	12	0	0	0	0.5	4.5	38	53	67.5
	24	4	18	24	25.5	41	57	60	57.5
	48	1	3	4	11	44	64.5	73.5	71.5
	96	6	14	15.5	20	37.5	53	59	58.5
	216	9	23	27.5	30.5	39	48	52	52.5
30° C.	12	1.5	7	8.5	16.5	46	62	73	74.5
	24	0	4.5	8	10.5	20.5	30	35.5	34
	48	12	21	29	33.5	45.5	52.5	55	57
	96	16	35	40	45.5	54.5	60	62	61
	216	3.5	11	17	24	39	49	54	56
35° C.	12	0	11.5	16.5	25.5	42	50.5	54.5	56
	24	2	11.5	16	21	36	53	62.5	64.5
	48	8.5	26	31	36.5	51	63	64.5	61.5
	96	12.5	36.5	36	48.5	57.5	64	61	58
	216	6.5	33.5	43	50.5	55.5	59	64.5	63.5
40° C.	12	1	4.5	6	10.5	28	49.5	62.5	64.5
	24	2	20	27.5	40.5	46	54	59.5	58
	48	5	26	27.5	34.5	48	56.5	61.5	59
	96	0.5	21	36	43	61	66.5	67	62
	216	0	12	18	31	46.5	54	56.5	57
50° C.	12	0	0.5	3	10	27	50	70.5	75.5
	24	0	0.5	2.5	18	43.5	63	73.5	77
	48	0	0	0	0.5	5.5	14.5	29	34.5
	96	0	0	0	0	0	0	0	0
	216	0	0	0	0	0	0	0	0
Unsoaked control.....		0	0.2	1.8	7.8	23.2	42.8	58.5	60.4

The control is the average of five unsoaked cultures.

indicate the contrary for asparagus seed. The seeds planted March 4, 1924 (table 7), germinated in soil, the temperature of which never exceeded 25° C., and remained below 20° C. most of the time. The highest germination obtained in the whole series was from seed soaked at 50° C. There were many cultures at all temperatures which were equal or superior to the control.

Laboratory tests (table 8) gave similar results.

TABLE 8

EFFECT OF THE TEMPERATURE OF SOAKING ON THE RATE OF GERMINATION AT LOW TEMPERATURES. SEEDS SOAKED AT 20° AND 30° C.; GERMINATED AT 20° C. IN THE LABORATORY.

Hours soaked	Temperature soaked	Percentage germination after:							
		3 days	4 days	5 days	6 days	8 days	10 days	15 days	17 days
144	20° C.	19	31	44	44	49	49	57	57
	30	19	32	41	42	47	50	50	51
120	20	9	21	35	38	40	49	49	49
	30	7	32	41	42	47	.....	50	51
96	20	10	23	34	36	38	39	41	43
	30	15	32	43	45	51	.....	.....	.....
72	20	1	10	19	32	36	39	46	46
	30	14	36	44	44	47	48	51	51
48	20	1	6	17	22	33	37	42	42
	30	9	23	31	40	43	44	46	46
24	20	0	0	5	10	17	21	26	26
	30	1	9	21	26	33	37	38	38
12	20	0	0	1	3	6	11	20	21
	30	0	0	8	11	18	21	29	30
Unsoaked control.....	.....	0	0	2	4	11	14	26	27

This table shows that asparagus seeds germinate faster even at a low temperature, if soaked before planting. Seeds soaked at 30° C. germinated more rapidly than seeds soaked at 20° C. even though both lots were germinated at 20° C. This difference in rate of germination becomes less marked with longer soaking. The final germination of asparagus seeds germinated at low temperatures is increased by soaking.

## V. SUMMARY AND CONCLUSIONS

1. In the case of *Asparagus officinalis* it is usually from two to six weeks, according to the soil and temperature conditions, before the seedlings appear above the surface. This delay may cause the grower much extra work while the plants are too small to cultivate.

2. That a period of dormancy does not occur in asparagus seed is shown by the fact that it germinates readily soon after harvesting.

3. The temperature at which untreated asparagus seed germinates most rapidly in the laboratory is between 25° and 30° C.

4. The rate of water intake by asparagus seed immersed in water at different temperatures (10° C. to 40° C.) is found to increase with the temperature of the water.

5. The maximum amount of water absorbed at any temperature is approximately 43 per cent of the original air dry weight.

6. Laboratory results show that the rate of germination of asparagus seed may be materially increased by various periods of soaking in water at different temperatures. Field results from duplicate cultures bear out these laboratory findings.

7. Asparagus seeds may be soaked for a period of nine days without reducing the final percentage of germination if the temperature does not exceed 40° C.

8. A reduction in final germination as compared with an unsoaked control may result from soaking at temperatures of more than 40° C.

9. In the laboratory tests asparagus seed soaked from two to nine days at temperatures of 20° to 38° C. germinate more quickly than seeds soaked for a shorter time under a similar temperature range. Those soaked at 25° C. to 35° C. germinated more quickly in general than those soaked at either higher or lower temperatures.

10. Laboratory and field data show that soaked seeds germinate more quickly than unsoaked seeds even though planted in cold soil.

11. For practical purposes a period of 3-5 days soaking at a temperature of 30° to 35° C. is recommended. This treatment is easily applied and the latitude of temperature and time conditions under which seed may be soaked without danger of injury makes the treatment simple and safe.

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THE RELATION OF THE SUBCUTANEOUS ADMIN-  
ISTRATION OF LIVING BACTERIUM ABORTUM  
TO THE IMMUNITY AND CARRIER PROBLEM  
OF BOVINE INFECTIOUS ABORTION

BY

GEORGE H. HART AND JACOB TRAUM

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\* C. M. Haring participated in the planning of this work and C. M. Carpenter in its actual prosecution from July 1, 1922, to June 30, 1923.

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The discovery of the etiological relationship of *Bacterium abortum* to bovine infectious abortion by Bang<sup>1</sup> in 1897 naturally led this investigator to turn his attention to experiments with the organism in producing immunity to the infection. In 1906 he<sup>2</sup> reported the experiments in which he was able to produce definite protective results in sheep, goats and cattle when he injected living organisms subcutaneously before pregnancy was established. On the other hand when the organisms had been killed by toluol before injection, little, if any, protection was afforded to the experimental animals.

Since that time McFadyean and Stockman<sup>14</sup> of England; Zwick, Zeller, Krage and Gminder<sup>23</sup>; Schermer and Ehrlich<sup>17</sup> of Germany; C. O. Jensen<sup>13</sup> of Denmark; Schroeder,<sup>15</sup> Smith and Little,<sup>18</sup> Birch and Gilman,<sup>3</sup> Hadley,<sup>8</sup> Huddleson,<sup>12</sup> Fitch and Boyd<sup>5</sup> in this country, and others have reported on the use of living and killed cultures of *Bacterium abortum* in attempts to immunize cattle against this organism. The work reported by most of these investigators consisted principally of observations on field trials under conditions in which there was no definite assurance that the treated and untreated animals picked up abortion infection and whether or not the amount of infection picked up perchance was approximately the same for both vaccinated and control animals. Other important, uncontrollable factors must have been present in these trials. In general, the results indicated that cattle injected when in a non-pregnant state with

living *Bacterium abortum* cultures produced a much higher percentage of apparently normal calvings than either the untreated animals or those which received killed organisms.

After the report made by Stockman<sup>20</sup> in 1914 of field observations on 2,150 cattle (1,279 of which were injected with live organisms, 121 with killed organisms and 750 remained as untreated controls), laboratories in many countries began the production and distribution of living abortion organisms as a vaccine for the control of bovine infectious abortion. The governments of England, Canada, South Africa, Sweden, Holland, Switzerland and others have been producing and distributing such vaccine to their stockmen. Many of the plants in the United States producing veterinary biologics are licensed by our federal Bureau of Animal Industry to manufacture and sell this product.

#### REASONS FOR THE INVESTIGATIONS

At the time we outlined our experiments some of the above-mentioned workers had not reported their results. We felt there was not sufficient information on the efficacy of live abortion organisms in the control of infectious abortion, and, rather than being a procedure to be carried out in a widespread manner in the field, it was still in the experimental stage. Even at this writing, many of the points included for investigation in our project are not satisfactorily answered by these investigators. Information based upon careful investigation regarding its efficiency in controlled experiments, the deleterious effect of its use on the vaccinated animals, the length of time the organisms remain viable in the animals, the effect on subsequent breeding, and other questions, had been so meager that general confidence in the method—and even justification for its use—was open to severe question in the minds of many investigators and livestock sanitary authorities. Frequently in this particular disease, curative and preventive measures used have been given credit for results which they did not deserve, because abortion tends to be self-limiting and may disappear without treatment. For this reason, only results from experiments which include control animals can be given very much weight.



## OUTLINE OF THE EXPERIMENTS

Our investigations were designed to furnish additional information on the important, and at that time still unsettled, question of the actual value of live abortion organisms in producing immunity. We also hoped to gain additional light on the localization, persistence, multiplication and elimination of the injected bacteria and to determine if it is necessary in the production of immunity in *Bacterium abortum* infection to have persistent multiplication and activity of the organism in the animal body or if the immunity is conferred upon an animal simply as the result of having been infected with the organism.

It was expected that the investigation would also show the extent to which the infection resulting from both the inoculation experiments to produce immunity and the ingestion experiments to produce infection would be injurious to the animals infected and also to animals associated with them.

*Source of the Cattle.*

For these purposes in the first series of experiments, 56 bovine females and 2 males were assembled. Fifteen of the females were taken from the University Dairy and five were of beef strain which had been in our possession for several years, having been originally purchased as young heifers for tuberculosis experiments but not used. All were known to be free from infection with *Bacterium abortum*. The remaining 36 females were dairy heifers purchased after a negative blood test from six herds with negative histories of abortion. The two bulls were obtained from one of the certified dairies where they had been raised. They were about fifteen months old at the time of purchase. In addition the bull at the University Dairy was used where mentioned. This animal had been with the dairy animals since August, 1917, and he, together with the other animals there, was free from *Bacterium abortum* infection as determined by extensive blood tests and milk examinations. The above animals were kept together for several months with the exceptions noted, and negative blood tests were obtained in all cases before the experiments began. They were divided into four groups as follows:

Group I, consisting of 20 animals, was the group used to determine the efficiency of the injection of live abortion organisms in preventing abortion.

Group II, consisting of 15 animals, constituted the controls, divided into two sub-groups:

A—Ten head to actually receive infectious material to produce abortion;

B—Five head left as association animals.

Group III, consisting of 10 animals, received vaccine but no further treatment in order to ascertain how long *Bacterium abortum* would remain in their bodies as a result of a single exposure by subcutaneous injection; also, to ascertain if the organism would be given off in the colostrum and placentae of these animals at the first subsequent parturition. The effect of the vaccination could be studied in these animals as well as in those of Group I.

Group IV, consisting of 11 animals, divided into two sub-groups:

A—Five head bred, without previous treatment, by the bulls after they had served the vaccinated animals in Groups I and III.

B—Six head left open so that in case opportunity offered they could be bred by the bulls shortly after breeding an aborting cow.

This group was intended to constitute a check on the possibility of exposed bulls transmitting the infection, through the medium of copulation, to non-infected females.

Table 1 gives the lists of these groups with the agglutination tests throughout the period covered by this report.

#### *Distribution of the Cattle.*

When the animals were divided into groups, they were placed in separate fields (fig. 1). The pastures used for the experiments comprise the north side of a cañon. The land is sloping and hilly and the drainage is in one direction from the hillside into the creek at the bottom of the cañon (from the upper to the lower part of fig. 1).

Drainage from the far-east pasture can, therefore, run through the east pasture and from the connecting pasture into the area occupied by the buildings and the road pasture. The remainder of the land drains directly into the creek. These facts were kept in mind in placing the animals so that infection from one group to another would not take place through the medium of drainage.

### Preparation of the Vaccine.

Four strains of the abortion organism were used in making the suspension. Two of these, A and 80, were old bovine laboratory strains which grew very rapidly and heavily on culture media. The third was a strain obtained from live abortion germ vaccine sold by a commercial firm in this country, and the fourth, 101, was a strain isolated (October 26, 1921) in this laboratory from an aborted bovine fetus. The cultures were grown on glucose glycerine bouillon and glucose glycerine agar, the growth on the latter being washed off with

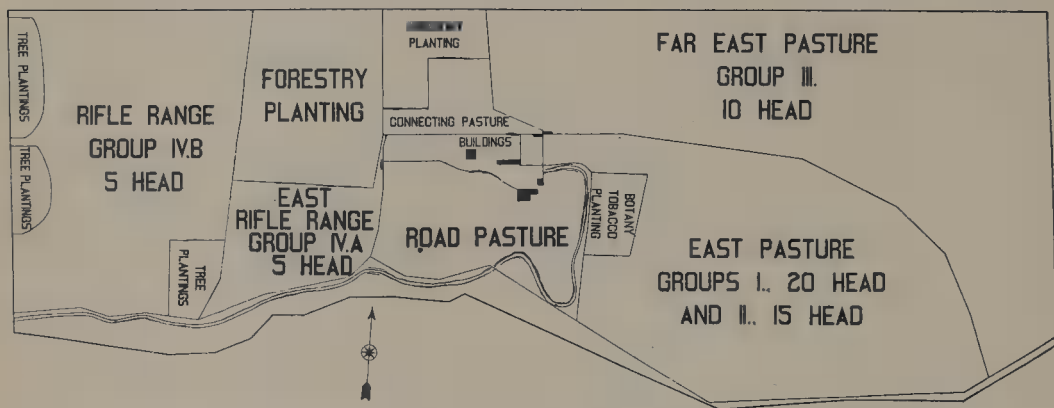


Fig. 1. Plot, plan of buildings and pastures in Strawberry Cañon, University of California, Berkeley, occupied by experimental cattle in abortion investigations.

saline solution and used to enrich the bouillon cultures. Subcultures were made and smears stained from each of the flasks which showed them to be pure cultures of the organism. All of the strains were known to be pathogenic for guinea pigs. The suspension of the organisms was tested with a silica comparator standard using Pear's precipitated fullers' earth. The technique of this preparation is given in the turbidity standard of the Standard Methods of Water Analysis by the American Public Health Association as used by Butterfield and Neill<sup>4</sup> in the Hygienic Laboratory in their work on various strains of meningococci.

In this work we desired to use organisms of known pathogenicity in a dose as high as that generally used in order that failure to produce protection could not be ascribed to lack of virulence or number of organisms. The silica comparator standard used had been prepared for meningococci. When the suspension of *Bacterium abortum* equalled the four billion per mil meningococci standard, it appeared as opaque as any of the samples of commercial abortion vaccine in

our possession and this was therefore the concentration used. When this was later tested with the Gates<sup>7</sup> opacimeter, it showed a reading of .9 cm. When the organisms in such a suspension were diluted and counted by the plate culture method, it yielded an average of eleven billion organisms per mil.

#### *Preparation of Infectious Material.*

Eight gallons of milk, from cows under observation by Hayes and Barger at the University Farm, Davis, were secured for use in this experiment. The milk from these cows was known to contain *Bacterium abortum*. To test this particular milk, 800 mls were centrifuged and the sediment inoculated intra-abdominally into guinea pigs 2334 and 2335. No. 2334 died soon after inoculation and was not autopsied. No. 2335 was killed at the end of eight weeks and found to have extensive lesions of *Bacterium abortum* infection. Its blood gave a positive agglutination test.

The following material from bovine fetuses, which had been received at the laboratory and found to contain *Bacterium abortum*, was mixed in salt solution to a volume of 1 gallon:

Fetus Number	Lungs	Stomach Contents	Intestinal Contents	On Ice Since
32	×	×	×	May 13
33	×	----	----	May 30
35	×	×	----	May 25
37	×	×	----	June 1
38	×	×	----	June 12
40	×	×	----	June 15

A bottle of 1-gallon capacity was used to hold 1250 mls of glycerine glucose broth culture of *Bacterium abortum*, strain 4, also the surface growth of strain 80 on 13 bottles of glycerine glucose agar washed off with salt solution. Strain 4 was isolated from the abscess of cow 4 following vaccination—80 was an old laboratory bovine culture and one of the strains used in the vaccine.

A third gallon bottle was used to hold 2000 mls of broth culture of strain 118 isolated in this laboratory March 10, 1922, from fetus 18.

A fourth gallon bottle was used to hold surface growth on fetus media agar, washed off with salt solution, of strains of *Bacterium abortum* recently isolated from fetuses 10, 20, 35, 37, 38, and 40.

A fifth gallon bottle was used to hold strains of *Bacterium abortum* on solid and liquid media isolated from guinea pigs inoculated with tissues of infected fetuses 3, 10, 11, and 18.

There were thus available 8 gallons of naturally infected milk, 1 gallon of infected fetus tissues in salt solution, and 4 gallon bottles containing cultures of *Bacterium abortum* and each filled to a gallon volume with water at the time of the infection, 6.30 to 9.30 P.M., June 26, 1922.

*Method of Preparing Colostrum and Placentae for Guinea Pig Inoculations from the Animals of All Groups.*

The colostrum for injecting the guinea pigs, as shown in table 2, was obtained in sterile pint jars immediately after calving. From 300 to 500 mils were taken in each case, some being collected from each of the four teats. This was brought to the laboratory and centrifuged in 100-mil centrifuge tubes for twenty minutes. Some of the fat from the surface and the sediment from the bottom of one or two tubes were mixed together and 1 to 2 mils injected intra-abdominally into each guinea pig.

The entire placenta, or as much of it as could be collected in each case, was placed in a sterile 1-gallon covered can and brought to the laboratory. In case it was soiled with manure or bedding, it was washed in tap water. It was then spread out on a tray and a careful examination made for any evidence of necrotic, hemorrhagic or other abnormal areas. Material for guinea pig injection was always taken from the most suspicious looking areas. This was ground in a mortar with sterile salt solution and injected intra-abdominally into guinea pigs.

Stained smears were also examined microscopically in each case.

EXPERIMENTAL DATA ON ANIMALS OF GROUP I

On February 7, 1922, the animals of this group were placed in the road pasture with those of Group III (fig. 1), and injected with the vaccine. Each animal was given subcutaneously, at one point on the left side of the neck, 20 mils of the material. The injected area was previously washed with a 3 per cent compound cresol solution as would be done in routine field practice.



The day after the injection a cold rain-storm began and continued four days. Practically all of the animals had a more or less marked reaction, probably exaggerated by the bad weather conditions. They stood humped up in the pasture and ate very little. Alfalfa hay was being fed to them at the time. By February 11, they were all eating normally and on the following day the weather had cleared and they appeared to have recovered from the effects of the vaccination. However, local swellings were present on all of the animals at the point of injection. In the majority of cases these increased in size for several weeks and, in a number of the cattle involved the prescapular lymph gland on the side injected. On March 10, 31 days after vaccination, the following conditions were found on examination of the injected areas:

- No. 4. Large abscess.
- No. 25. Large, soft abscess, 4" by 6" by 3". Opened by incision and material taken for culture.
- No. 403. Normal.
- No. 404. Enlarged gland.
- No. 405. Normal.
- No. 407. Normal.
- No. 408. Gland enlarged and hard.
- No. 410. Enlarged gland.
- No. 414. Soft abscess.
- No. 415. Small abscess.
- No. 418. Enlarged gland.
- No. 421. Abscess had opened naturally.
- No. 424. Enlarged gland.
- No. 426. Abscess had opened naturally.
- No. 428. Large abscess, 6" by 4". Opened.
- No. 433. Slight swelling.
- No. 434. Abscess had opened naturally.
- No. 2182. Large abscess.
- No. 2305. Gland slightly enlarged.
- No. 2314. Abscess had opened naturally.

These animals at that time were not in such good condition as the 13 controls in Group II. The pus from the abscesses was identical in all cases, being thick yellowish-white in appearance. This condition probably would have been avoided to a considerable extent had the suspension of the organisms been further diluted and the injection made in several areas instead of placing the entire 20 mils at one point. Pus was collected from the abscesses on cows 4 and 25. Inoculations made from this material developed pure cultures of

*Bacterium abortum* from cow 4 and *Bacterium abortum* with some contamination from cow 25.

On February 21, 1922, fourteen days after vaccination, blood samples were taken from these animals and all gave a positive reaction to the agglutination test (see table 1).

The breeding of these animals was begun April 10, 1922, sixty-two days after the vaccination, when the bulls were removed from Group II and kept corralled so that breeding dates could be secured.

The animals in this group, although they had apparently entirely recovered from the effect of the vaccination, came in heat slowly. The following breeding took place:

- Bull 412 bred on April 18, 1922, to no. 433.
- Bull 412 bred on April 19, 1922, to no. 434.
- Bull 412 bred on April 20, 1922, to no. 2314.
- Bull 412 bred on April 20, 1922, to no. 403.
- Bull 412 bred on April 22, 1922, to no. 408.
- Bull 412 bred on April 26, 1922, to no. 421.
- Bull 412 bred on April 27, 1922, to no. 428.
- Bull 412 bred on April 27, 1922, to no. 2182.
- Bull 412 bred on May 3, 1922, to no. 410.
- Bull 412 bred on May 11, 1922, to no. 424.
- Bull 411 bred on April 21, 1922, to no. 25.
- Bull 411 bred on April 27, 1922, to no. 407.
- Bull 411 bred on April 29, 1922, to no. 414.
- Bull 411 bred on April 29, 1922, to no. 434.
- Bull 411 bred on May 1, 1922, to no. 418.
- Bull 411 bred on May 19, 1922, to no. 405.

When the breeding of this group was begun, these animals, with those of Group III, were placed in the east pasture where there was good green feed. After breeding, each animal was removed to the road pasture.

On May 12, 1922, on account of the animals breeding slowly and time being an important factor owing to the control animals (Group II) being pregnant, a rectal examination was made of the unbred animals. The ovaries were massaged and the corpora lutea were expressed from nos. 404, 405, and 426.

On June 24, 1922, the 20 animals of Group I were separated from the ten of Group III and kept in the road pasture. They had all been bred, but on this date they were examined for pregnancy. Some of them had been too recently bred for this to be of any value. The result of the examination is shown below:

No. 4.	Bred April 19.	Pregnant.
No. 25.	Bred April 21.	Pregnant.
No. 403.	Bred April 20 and June 3.	?
No. 404.	Bred June 20.	?
No. 405.	Bred May 19 and June 19.	?
No. 407.	Bred April 27.	Pregnant.
No. 408.	Bred April 22.	Pregnant.
No. 410.	Bred May 3.	Pregnant.
No. 414.	Bred April 29.	Pregnant.
No. 415.	Bred June 10.	?
No. 418.	Bred May 1.	Pregnant.
No. 421.	Bred April 26 and June 3.	?
No. 424.	Bred May 11.	?
No. 426.	Bred June 24.	?
No. 428.	Bred April 27.	Pregnant.
No. 433.	Bred April 18.	Pregnant.
No. 434.	Bred April 29.	Pregnant.
No. 2182.	Bred April 27.	Pregnant.
No. 2305.	Bred May 25.	?
No. 2314.	Bred April 20.	Pregnant.

A rectal examination only was made as most of them were heifers and to get the hand into the vagina was difficult or impossible. Also we<sup>21</sup> hold the opinion that under certain unrecognized conditions, bimanual examination may be the cause of abortion in a small percentage of cases.

In this group, cows 405, 415 and 428 were later found not to have conceived. No. 428 on June 24 was thought to be pregnant, having been bred on April 27, fifty-eight days prior to the examination. It is believed that this diagnosis of pregnancy was in error rather than that she aborted, since she was being daily observed with other animals in the group and was seen to be in heat on August 5.

This group of animals was kept corralled from June 24 to 26 with the animals of Group II-A. This was done to control their water supply in the hope that they would drink from the watering-trough the infectious material to be given them on the latter date.

The administration of the infectious material was delayed until late in the day in order that it would not be exposed to strong light during the process. An effort was made to mix the material in the drinking water. On account of discoloration of the water by the milk and a slight odor from the fetus material, the animals would not drink, although they had had little water for the previous forty-eight hours. They were then placed in the chute and drenched.

Five drenching batches were made by taking 500 mls from each of the five 1-gallon bottles and 500 mls of milk, making a total of 3000 mls, of which mixture each cow was drenched with approximately 1 pint. The remainder of the infectious mixture was placed in the watering-trough, baled alfalfa hay was opened and the flakes were soaked in the trough until the solution was absorbed. It was then spread around the corral for the animals to eat. They had not been previously fed on that day and no difficulty was experienced in getting them to eat the hay. The next morning the animals were turned into the east pasture. The watering-trough in the corral was disinfected and no further infection was given to the cattle.

*History of First Pregnancy of Animals of Group I, Vaccinated and Infected.*

The parturition history of the 20 animals in Group I, which were kept with the animals in Group II after infection and subjected to the same conditions except that the former had been vaccinated with live abortion organisms seventy days or longer prior to breeding, is given in table 2.

From an examination of the data it will be seen that every one of the 17 animals in this group that became pregnant carried their calves to term except no. 407, which was accidentally killed in the last month of gestation. Pregnancy was progressing normally and no evidence of abortion infection could be found in her tissues, the results of the examination of which will be discussed later.

The following examination and notes were made of the fetus:

Fetus of cow 407: Removed from uterus after death of dam caused by broken neck; black and white female; 8 months gestation.

Externally: Normal.

Internally: Tissues normal.

Heart: Few petechial hemorrhages on myocardium of ventricles.

Stomachs: Distended with a viscid, faintly-clouded fluid, which showed no evidence of being stained with meconium.

Rectum: Meconium made up of firm, mucous-coated pellets, greenish in color.

Cultures:

Heart's blood,	negative
Lung,	negative
Liver,	negative
Spleen,	negative
Stomach contents,	negative
Small intestine,	negative
Meconium rectum,	negative

TABLE 2.—BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM

## FIRST PREGNANCY

No. of Animal	Breeding date	Calving date	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Post mortem findings Guinea pigs	Cultures from spleens of Guinea pigs	Blood reaction of Guinea pigs	Placenta
<b>GROUP I</b>									
4	Apr. 19, 1922	Jan. 23, 1923	2848-49	2846-47	Mar. 14, 1923	2846 + Others —	2846 + Others —	2846 + Others —	Expelled normally
25	Apr. 21, 1922	Feb. 7, 1923	2923-24	2921-22	Mar. 26, 1923	—	—	—	Expelled normally
403	Apr. 20, 1922; June 3, 1922	Mar. 4, 1923	3015-16	3013-14	Apr. 15, 1923	—	—	—	Expelled normally
404	June 20, 1922	Apr. 1, 1923	3123-24	3125-27	May 16, 1923	—	—	—	Expelled normally
405	May 19 and June 19, 1922.								
	Did not conceive.								
407	Apr. 27, 1922	Died	2782	.....	Feb. 13, 1923	—	—	—	
		Dec. 28, 1922							
408	Apr. 22, 1922	Jan. 25, 1923	2864-65	2862-63	Mar. 15, 1923	2863 + Others —	2863 + Others —	2863 + Others —	Expelled normally
410	May 3, 1922	Feb. 2, 1923	2889-90	2891-92	Mar. 16, 1923	—	—	—	Expelled normally
414	Apr. 29, 1922	Feb. 9, 1923	2934-35	2932-33	Mar. 26, 1923	—	—	—	Expelled normally
415	June 10, 1922. Did not conceive.								
418	May 1, 1922	Feb. 2, 1923	2893-94	2895-96	Mar. 16, 1923	—	—	—	Expelled attached to calf
421	Apr. 26, 1922; June 3, 1922	Mar. 6, 1923	3025-26	3023-24	Apr. 25, 1923	3023-24 + Others —	3023-24 + Others —	3023 + Others —	Expelled normally
424	May 11, 1922	Feb. 7, 1923	2927-28	2925-26	Mar. 26, 1923	—	—	—	Expelled normally
426	June 24, 1922	Mar. 24, 1923	3066-67	3080-81	3066 died Mar. 26, 1923 Others killed May 4, 1923	3080-81 + Others —	3080-81 + Others —	3080-81 + Others —	Expelled normally
428	Apr. 27, 1922. Did not conceive.								
433	Apr. 18, 1922	Jan. 20, 1923	2834-35	2836-37	Mar. 1, 1923	—	—	—	Calved 9 A.M. Placenta not passed at 11 A.M. Removed and some cotyledons adherent.
434	Apr. 29, 1922	Feb. 2, 1923	2904-05	2902-03	Mar. 19, 1923	—	—	—	Expelled normally
2182	Apr. 27, 1922	Jan. 23, 1923	2860-61	2858-59	Mar. 15, 1923	—	—	—	Expelled normally
2305	May 25, 1922	Feb. 27, 1923	2995-97 2999-3000	2985- 2985- 2985- 2985-	2995-97-99 died. Others killed Apr. 15, 1923 Mar. 1, 1923	—	—	—	Retained
2314	Apr. 20, 1922	Jan. 20, 1923	2838-39	2840-41	Mar. 1, 1923	—	—	—	Expelled normally





TABLE 2.—BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM—(Continued)

FIRST PREGNANCY

No. of Animal	Breeding date	Calving date	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Post mortem findings Guinea pigs	Cultures from spleens of Guinea pigs	Blood reaction of Guinea pigs	Placenta
GROUP III									
402.....	Did not get pregnant.								
406.....	Did not get pregnant.								
413.....	Apr. 10, 1922.....	Jan. 17, 1923	2826-27	2824-25	2825 died Others killed Mar. 1, 1923	—	None made	—	Expelled normally
419.....	Did not get pregnant.								
2297.....	After June 24, 1922.....	Apr. 7, 1923	3140-41	3138-43	3140-41 died 3138-43 killed May 24, 1923	—	—	—	Expelled normally
2313.....	Did not get pregnant.								
2315.....	Did not get pregnant.								
2318.....	After June 24, 1922.....	Apr. 6, 1923	3134-35	3132-33	May 24, 1923	—	—	—	Expelled normally
2319.....	Did not get pregnant.								
2321.....	After June 24, 1922.....	May 14, 1923	3223-24	3221-22	June 29, 1923	—	—	—	Expelled normally
GROUP IV									
435.....									
437.....									
438.....									
439.....									
445.....									
440.....									
441.....									
442.....									
443.....									
444.....									
446.....									

Not in experiment the first year.

TABLE 2.—BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM—(Continued)

## SECOND PREGNANCY

No. of Animal	Breeding date	Calving date	No. of Calf	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Blood Reaction of Guinea pigs	Post mortem Guinea pigs	Cultures from spleens of Guinea pigs	Placenta
<b>GROUP I</b>										
4.....	May 14, 1923	Feb. 22, 1924	A. E. 52	3673-74	3675-76	3674 died Others killed Apr. 4, 1924 Apr. 24, 1924	3676 Susp. Others —	3676 Susp. Others —	3675-76 + Others —	Manually removed—not adherent. Metritis followed. Expelled normally
25.....	May 14 and 26, 1923	Mar. 9, 1924	A. E. 61	3724-35	3722-23		—	—	—	
403.....	Apr. 25, 1924	Second pregnancy	n	not yet termi	nated. Pre	gnant to April	breeding.			
404.....	Aug. 2, 1923	May 14, 1924	A. E. 72	3846-47	3848-49	July 2, 1924	—	—	—	Expelled normally
405.....	May 14, 1923	Feb. 20, 1924	A. E. 50	3652-53	3650-51	Apr. 3, 1924	—	—	—	Expelled normally
407.....	Died during first pregnancy									
408.....	May 12, 1923	Feb. 23, 1924	A. E. 53	3679-80	3677-78	3680 died Mar. 21, 1924 Others killed Apr. 8, 1924	3677 + Others —	3677 + Others —	3677 + Others —	Expelled normally
410.....		Second pregnancy	n	not yet termi	nated. Def	initely pregna	nt.			
414.....		June 22, 1924	A. E. 74	3908	3907	Aug. 13, 1924	.....	.....	.....	Expelled normally
415.....		Mar. 4, 1924	A. E. 60	3709-10	3707-08	Apr. 18, 1924	—	—	—	Expelled normally and eaten
418.....	Apr. 25, 1924	Second pregnancy	n	not yet termi	nated. Pre	gnant to April	breeding.			
421.....		Killed at end of first pregnancy.								
424.....		Killed at end of first pregnancy.								
426.....	June 12, 1923	Mar. 19, 1924	A. E. 64	3750-51	3748-49	May 5, 1924	—	—	—	Expelled normally
428.....		Only animal in experiment which never became pregnant.								
433.....	May 3, 1923	Feb. 8, 1924	A. E. 45	3584-85	3586-87	3584 died Feb. 10, 1924 Others killed Mar. 21, 1924	—	—	—	Expelled normally
434.....	July 23, 1923	May 2, 1924	A. E. 70	3818-19	3816-17	Mar. 24, 1924	—	—	—	Expelled normally
2182.....	May 2, 1923	Feb. 11, 1924	A. E. 48	3590-91	3588-89	Mar. 24, 1924	—	—	—	Manually removed. In part adherent.
2305.....	Dec. 14, 1923	Oct. 4, 1924	A. E. 76	4071-72	4069-70-73	4071-72-70 died. Others killed Nov. 18, 1924 Mar. 21, 1924	—	—	—	Expelled normally
2314.....	May 3, 1923	Feb. 8, 1924	A. E. 47	3580-81	3582-83		—	—	—	Manually removed. Adherent.

TABLE 2.—BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM—(Continued)  
SECOND PREGNANCY

No. of Animal	Breeding date	Calving date	No. of Calf	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Blood Reaction of Guinea pigs	Post mortem Guinea pigs	Cultures from spleens of Guinea pigs	Placenta
GROUP II										
20.....	Feb. 12, 1923	Nov. 20, 1923	A. E. 42	3483-84	3481-82	Jan. 8, 1924	3481-82 + Others —	3481-82 + Others —	3481-82 + Others —	Expelled normally
26.....	May 23, 1923	Mar. 4, 1924	A. E. 59	3703-04	3705-06	Apr. 18, 1924	—	—	—	Expelled normally
401.....	May 19, 1923	Feb. 28, 1924	A. E. 55	3685-86	3687-88	Apr. 11, 1924	3687-88 + Others —	3687-88 + Others —	3687-88 + Others —	Expelled normally
416.....	Feb. —, 1923	Nov. 13, 1923	A. E. 41	3447-48	3445-46	Jan. 7, 1924	+	+	+	Manually removed but not adherent
429.....	Apr. 7, 1923	Jan. 3, 1924	A. E. 43	3539-40	3537-38	3538 died Jan. 17, 1924 Others killed Feb. 14, 1924	3537 + Others —	3537 + Others —	3537 + Others —	Expelled normally
431.....	May 27, 1923	Oct. 22, 1923	A. E. 40	3428-29	.....	Dec. 11, 1923	—	—	None made	Expelled normally
2060.....	May 14 and	Unable to get pregnant	to get pregnant	3814-15	3812-13	June 14, 1924	3812-13 + 3814-15 —	3812-13 + 3814-15 —	3812-13 + 4814-15 —	Expelled normally
2180.....	July 22, 1923	Apr. 27, 1924	A. E. 69	3644-45	3646-47	Apr. 3, 1924	3646-47 + Others —	3646-47 + Others —	3646-47 + Others —	Expelled normally
2181.....	May 13, 1923	Feb. 19, 1924	A. E. 49	3559-60	3557-58	Mar. 13, 1924	—	—	—	Manually removed but not adherent
2312.....	Apr. 25, 1923	Jan. 31, 1924	A. E. 44	3689-90	3691-92	Apr. 11, 1924	—	—	—	Manually removed but not adherent
183.....	May 13, 1923	Feb. 29, 1924	A. E. 56	3853-54	3855-56	July 2, 1924	—	—	—	Expelled normally
430.....	Aug. 6, 1923	May 17, 1924	A. E. 73	4076-77	4074-75	Nov. 18, 1924	—	—	—	Expelled normally
436.....	Dec. 25, 1923	Oct. 6, 1924	A. E. 77	3683-84	3681-82	Apr. 8, 1924	3681-82 + Others —	3681-82 + Others —	3681-82 + Others —	Expelled normally
2298.....	May 14, 1923	Feb. 25, 1924	A. E. 54	3671-72	3669-70	Apr. 4, 1924	—	—	—	Expelled normally
2317.....	May 9, 1923	Feb. 20, 1924	A. E. 51							

TABLE 2.—BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM—(Concluded)  
SECOND PREGNANCY

No. of Animal	Breeding date	Calving date	No. of Calf	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Blood Reaction of Guinea pigs	Post mortem Guinea pigs	Cultures from spleens of Guinea pigs	Placenta
<b>GROUP III</b>										
402.....	June 1, 1923	Mar. 22, 1924	A. E. 66	3759-60	3757-58	May 5, 1924	—	—	.....	Expelled normally
406.....	June 13 and Sept. 20, 1923	June 26, 1924	A. E. 75	Placenta lost	3909-10	Aug. 13, 1924	—	—	—	Expelled normally
413.....	.....	Killed at end of first pregnancy.								
419.....	June 10 and July 2, 1923	Apr. 5, 1924	A. E. 67	3765-66	3767-68	May 19, 1924	—	—	—	Expelled normally
2297.....	.....	Killed at end of first pregnancy.								
2313.....	May 9, 1923	Feb. 8, 1924	A. E. 46	3576-77	3578-79	3576-77 Mar. 18, 1924 3578-79	—	—	—	Manually removed but not adherent
2315.....	June 2, 1923	Mar. 12, 1924	A. E. 62	3730-31	3728-29	Mar. 21, 1924	—	—	—	Expelled normally
2318.....	June —, 1923	Mar. 13, 1924	A. E. 63	3738-39	3736-37	Apr. 24, 1924	—	—	—	Expelled normally
2319.....	June 29, 1923	Apr. 12, 1924	A. E. 68	3769-70	3771-72	May 30, 1924	—	—	—	Expelled normally
2321.....	June —, 1923	Mar. 19, 1924	A. E. 65	3746-47	3744-45	May 5, 1924	—	—	—	Expelled normally
<b>GROUP IV</b>										
435.....	Dec. —, 1922	Oct. 6, 1923	A. E. 38	None injected	None injected	.....	.....	.....	.....	Expelled normally. Lost.
437.....	Dec. —, 1922	Died Sept. 26, 1923	Ruptured uterus	3381-82	.....	Died Sept. 28, 1923	—	—	—	Manually removed but not adherent
438.....	Mar. 24 and May 21, 1923	Mar. 3, 1924	A. E. 58	3701-02	3699-3700	Apr. 18, 1924	—	—	—	Expelled normally
439.....	Aug. 1, 1923	May 10, 1924	A. E. 71	3829-30	3827-28	June 20, 1924	—	—	—	Expelled normally
445.....	May 27, 1923	Mar. 2, 1924	A. E. 57	3695-96	3697-98	Apr. 18, 1924	—	—	—	Expelled normally
440.....	.....	Not used in experiment.	in experiment.							
441.....	.....	Not used in experiment.	in experiment.							
442.....	Jan. 2, 1923	Oct. 9, 1923	A. E. 39	None injected	None injected	.....	.....	.....	.....	Expelled normally and partly eaten
443.....	.....	Not used in experiment.	in experiment.							
444.....	Apr. 7, 1923	Jan. 11, 1924	.....	3545-46	3543-44	3545-46 died Jan. 13, 1924 3543-44 Others killed Feb. 28, 1924	—	—	—	Expelled normally and partly eaten
446.....	.....	Not used in experiment.	in experiment.							



Blood serum of the calf was negative to the agglutination test.

Smears made from stomach contents were negative.

Guinea pig 2783: Injected with stomach contents; killed February 12, 1923; negative.

Guinea pig 2774: Injected with extract of the lung, liver and spleen; killed February 13, 1923; negative.

All of the animals in this group passed their placentae normally except nos. 433 and 2305. The former calved at 9 A.M. and since the placenta was desired for examination, it was manually removed at 11 A.M. Some of the cotyledons in the apex of the pregnant born were markedly adherent. This afterbirth, however, might have passed normally had more time been given.

No. 2305 calved at 11 A.M., with assistance from the attendant, after having been in labor since 7 A.M. This was a small heifer and the calf was large and expelled dead. Postmortem examination showed the lungs had not been inflated. While it was an anterior presentation, death may have occurred during parturition or may have resulted from inflammation of the placenta which was present. The following day at 11 A.M. part of the placenta was protruding from the vagina and was torn off and placed in a sterile can by the attendant. At 2 P.M. a quantity sufficient to nearly reach the floor had been ejected. On removing this and making a manual examination, shreds of tissue were found to be still adherent to the maternal cotyledons and there was considerable discharge from the uterus. This was, therefore, a definite case of retained placenta. Three days later the heifer was again examined and shreds of the placenta were still found to be attached to the uterus.

The first two guinea pigs inoculated with placental material died in forty-eight hours. Two others were then inoculated with uterine exudate. One of these also died in forty-eight hours but the other lived. This latter was finally killed at the end of six weeks and was negative for *Bacterium abortum*.

The following examination notes were made of this calf:

Expelled dead from dam 2305, 11 A.M., February 27, 1923; apparently mature and well developed; black and white; female.

Externally: Normal.

Internally: Tissues appeared normal.

Heart: Base of ventricle heavily spotted with petechial hemorrhages.

Lungs: Normal, not inflated.

Liver, Spleen and Kidney: Normal.

Stomachs: Filled with a clear mucus which was normal.

Intestines: Showed normal meconium.

Cultures:

Heart's blood,	negative
Lung,	negative
Spleen,	negative
Liver,	negative
Stomach contents,	negative
Small intestine,	negative
Large intestine,	negative
Meconium rectum,	negative

Guinea pig 2983: Injected with extract from lung, liver, spleen; killed April 15, 1923; negative.

Guinea pig 2984: Injected with stomach contents; killed April 15, 1923; negative.

Smears:

Lung,	negative
Stomach contents,	negative

The only other calf deserving mention was from no. 418. This calf was expelled with the placenta and the umbilical vessels remained intact. Birth occurred about 5 A.M. and the calf was not found by the attendant until 6 A.M. It was alive but very dull. The umbilical vessels were severed and the calf died about one-half hour later.

The following examination and notes were made of this calf:

Sex: Female.

Color: Black and white.

Born: February 2, 1923, to dam 418.

Externally: Normal.

Internally: Tissues appeared normal.

Lungs: Perfectly inflated.

Stomachs: Filled with a faintly clouded mucus, which was apparently normal.

Intestines: Showed normal meconium.

Cultures:

Heart's blood,	negative
Lung,	negative
Spleen,	negative
Liver,	negative
Stomach contents,	negative
Small intestine,	Gram negative slender rod; <i>B. coli</i>
Meconium rectum,	negative

Blood serum: Negative to the agglutination test.

Guinea pig 2897: Injected with extract from lung, liver, spleen; died February 13, 1923; *B. coli* in heart's blood; lungs congested.

Guinea pig 2898: Injected with stomach contents; killed March 19, 1923; negative.

It will be observed (table 2) that all of the placentae of Group I were negative for *Bacterium abortum*, while samples of the colostrum from four of the animals contained the organism. This suggests that vaccinated animals are not very liable to expel the organism from the genital tract at parturition following vaccination even when exposed to severe infection during pregnancy. These experiments confirm the fact that in persistent carriers, the udder is the seat of the infection. No conclusion can be drawn, however, as to whether the udder infection in these four cases resulted from the vaccination or from the infection by ingestion. It should be observed in this connection that none of the animals of Group III which were vaccinated only, eliminated *Bacterium abortum* with their colostrum or placentae.

#### *History of Second Pregnancy of Animals of Group I.*

After the first pregnancy in these animals, one, no. 407, died and two others, nos. 421 and 424 were killed. This left 17 animals in the group for study in the second pregnancy, three of which had failed to get with calf the first year. Two of these, nos. 405 and 415, were successfully bred the second year. The remaining animal, no. 428, we were unable to get with calf. Her genital tract clinically seemed to be normal. She came in heat regularly and from May 10, 1923, to February 13, 1924, she was bred ten times without result and was slaughtered February 18, 1924.

The breeding and calving history of the second pregnancy in this group, together with guinea pig inoculations of the placentae and colostrum, are given in table 2.

Three of the 17 animals, 403, 410 and 418, have not as yet calved the second time, but are definitely with calf.

Only two animals, nos. 4 and 408, in the group eliminated *Bacterium abortum* at the termination of the second pregnancy, whereas four had done so at the termination of the first pregnancy. In both cases it was in the colostrum. These animals, nos. 4 and 408, had given a similar result in their first pregnancies. No. 421, a third animal showing infected colostrum in the first pregnancy, had been killed shortly after the first parturition. The remaining animal, no. 426, in this group eliminating *Bacterium abortum* at the end of the first pregnancy, was negative in the second.

## EXPERIMENTAL DATA ON ANIMALS OF GROUP II

On February 7, 1922, the 13 animals of Group II, A and B, were given a final examination for pregnancy preparatory to placing them in the far east pasture (fig. 1) with the two bulls. One animal, no. 401, was found on rectal examination to be in early pregnancy, a fact not recognized at the time of purchase. One dairy animal, no. 2060, was definitely known to be pregnant to the dairy bull. The remaining eleven were not pregnant. Two other animals were added later to make fifteen, the desired number.

On April 10, 1922, the bulls were taken from this group. During the sixty-two days that they were with them, eight of the eleven open animals became pregnant. Nos. 183, 2317 and 430 did not become pregnant and were later bred to the dairy bull, conceiving without difficulty. It is probable that they did not come in estrum during the sixty-two day period since feed conditions in the pasture were not very good and the weather was cold and rainy.

On June 24, 1922, the ten animals of Group II-A were examined per rectum and found to be definitely pregnant. Nos. 183, 430 and 2317 were at the dairy for breeding to the dairy bull and were later returned with nos. 2298 and 436 to constitute the five association animals of Group II-B.

The ten pregnant animals of Group II-A were moved on this date from the far-east pasture to the road and east pastures with the animals of Group I.

They were kept corralled from June 24 to 26 to control their water supply in the hope they would drink from the watering-trough the infectious material to be given them on the latter date.

These animals were infected on June 26 in the identical manner as those of Group I and were turned into the east pasture with them the next day.

On July 10, fourteen days after infection, blood was drawn from these animals and with the possible exception of no. 401, all gave a positive agglutination test, although they had all given continuously negative reactions prior to the time of infection on June 26. This indicated that they had been infected with the *Bacterium abortum* by the method used (see table 1).

TABLE 3.—PARTURITION HISTORY OF TEN CONTROLS IN GROUP II AFTER INFECTION BY INGESTION, JUNE 26, 1922

Number	Aborted	Calved	Placenta		Fetus		Colo- strum	Guinea pig inoculation for Bacterium abortum	
			Smears	Guinea pig	Cul- tures	Guinea pig			
2060	.....	July 6, 1922.....	—	.....	.....	.....	—	—	No sample taken
401	.....	July 23, 1922.....	+	+	.....	.....	+	—	—
2181	Aug. 21, 1922—5 months fetus.....	.....	+	+	.....	.....	.....	+	—
2180	Sept. 2, 1922—6 months fetus.....	.....	+	+	+	+	+	+	—
429	Sept. 7, 1922—6½ months fetus.....	.....	.....	+	+	+	+	+	—
416	Sept. 7, 1922—6 months fetus.....	.....	+	+	+	+	+	+	—
20	Sept. 10, 1922—5½ months fetus.....	.....	—	+	—	—	+	—	—
431	Sept. 20, 1922—5 months fetus.....	.....	—	+	+	+	+	+	—
2312	.....	Dec. 3, 1922.....	—	—	.....	.....	—	Dry—advanced pregnancy...	Pregnant—No sample taken
26	.....	Dec. 3, 1922.....	—	—	.....	.....	—	Dry—advanced pregnancy...	Pregnant—No sample taken



*History of First Pregnancy of Infected Animals, Group II-A.*

Table 3 gives data on the existing pregnancy in these ten control animals at the time infection was given by the mouth.

It will be observed from examination of this table that six of the ten animals aborted from 56 to 86 days following the infection. No. 2060 calved normally ten days after the infection, which was too soon for it to have caused abortion. No. 401 calved 27 days after the infection—the calf was weak but lived. The placenta was retained and on removal and examination, abortion organisms were found in great numbers in smears and cultures, and inoculated guinea pigs were positive. They were also present in the colostrum. The existing pregnancies of nos. 2312 and 26 were apparently not affected by the infectious material and both animals calved normally on the same date, 160 days after the infection. The examination of the agglutination reaction of these two animals (table 1) shows quite definitely that they became infected but overcame it and remained entirely negative to the agglutination test.

Two animals in this group, nos. 2060 and 401, were much farther advanced in pregnancy at the time of infection than any animals in Group I. The six animals that actually aborted, however, were only about one month farther advanced than a number of animals of Group I. The bulls were with the animals of Group II from February 7 to April 10 and then turned with those in Group I, a number of which were bred during April. We do not think, therefore, that this difference in the period of gestation had any marked effect on the results obtained.

*History of First Pregnancy of Association Animals Group II-B.*

Four of these animals bred to the dairy bull had been at the University Dairy during the infection period and the fifth, purchased in early pregnancy, was not brought on the premises until August, 1922, with the animals for Group IV. They were added to the other animals of Groups I and II on the following dates: Nos. 183 and 2298 on July 25, 1922; nos. 2317 and 430 on August 10, 1922; and no. 436 on September 26, 1922.

The parturition data on these animals are given in table 2.

The six abortions in the ten infected controls of Group II-A, actually took place between August 21 and September 20, 1922. The association animals were in direct contact with them during all of

this period except no. 436, which was added six days after the last abortion occurred. This was the only animal of the five that escaped infection although all of them carried their calves to term.

In studying the agglutination tests (table 1) of these animals, it is interesting to note how the agglutination titre of no. 2298 gradually increased and that of no. 183 remained entirely negative. This latter animal furnishes an example of how the agglutination test may fail to detect a spreader of the organism. No. 430 gave very slight indication of reaction to the agglutination test and no. 2317, although showing much better evidence of infection in the tests made November 10, 1922, March 23 and July 11, 1923, did not at any time develop a definitely positive reaction.

#### *History of Second Pregnancy of Animals, Group II-A and B.*

Following the first pregnancy in this group all of the animals were successfully bred the second time except no. 2060. This animal had previously calved July 6, 1922, ten days after receiving the infectious material by the mouth and too soon for abortion to have occurred. Thereafter although she came in heat frequently and was bred on nine occasions between April 23, 1923, and January 25, 1924, she failed to get with calf and was killed February 28, 1924. Her genital tract at time of slaughter was apparently normal. The examination of her tissues yielded negative results.

No. 431 died October 22, 1923, from traumatic pericarditis when five months pregnant. The vagina was ligated and the uterus removed with enclosed fetus. This material was brought to the laboratory and the following examination made:

Fetus: Five months gestation. Normal.

Cultures:

Cotyledons

Heart blood

Lung

Liver

Spleen

Stomach contents

Intestinal contents

Meconium rectum

All cultures developed a growth of a Gram positive, sporulating rod (*B. subtilis*)

Guinea pigs:

3428, 3429, injected with placental extract.

3424, injected with meconium rectum.

3425, injected with lung, liver and spleen extract.

3426, injected with stomach and intestinal contents.

The guinea pigs were killed December 11, 1923, and all were found to be in a normal condition.

The remaining eight infected control animals (A) that had received infectious material and the five association animals (B) carried their calves to term.

Nos. 26 and 2312 of the infected controls (A) again failed to show *Bacterium abortum* in the colostrum or placentae at this parturition. The colostrum of all the other six was positive. Of these only no. 401, the animal to calve twenty-seven days after the infectious material was given in 1922, showed infection of the placenta at the second parturition.

The five association animals (B) were negative except the colostrum of no. 2298. It is interesting that the single animal in this group that remained a carrier of the infection was the only one to show a definitely positive blood reaction (see table 1).

#### EXPERIMENTAL DATA ON ANIMALS OF GROUP III

On February 7, 1922, the animals of this group were vaccinated in the same manner as those of Group I and placed in the road pasture with them. They were affected by the vaccination in a similar manner to the animals of Group I.

On March 10, thirty-one days after vaccination, the following condition was found on examination of the injected area in these animals:

- No. 402. Abscess had opened naturally.
- No. 406. Enlarged gland.
- No. 413. Enlarged gland.
- No. 419. Enlarged gland.
- No. 2297. Enlarged gland.
- No. 2313. Abscess had opened naturally.
- No. 2315. Enlarged gland.
- No. 2318. Enlarged gland.
- No. 2319. Normal.
- No. 2321. Normal.

These animals at the time were not in such good condition as the thirteen controls of Group II.

On February 21, 1922, fourteen days after vaccination, blood samples were taken from these animals and all gave a positive reaction to the agglutination test (see table 1).

The breeding of this group was begun April 10, 1922, sixty-two days after vaccination, when the bulls were removed from Group II and kept corralled so that breeding dates could be secured. On the night of April 10 the bulls were turned out with the cattle and no. 413 was bred. The animals came in heat slowly although they had apparently entirely recovered from the effects of the vaccination. The following breeding took place:

Bull 412 bred on April 23, 1922, to no. 2297.

Bull 411 bred on April 27, 1922, to no. 406.

While this breeding was going on, the animals of this group were still with those of Group I and were on good green feed in the east pasture.

On May 12, 1922, while examining the genital tracts of the unbred heifers of this group, no. 2318 was found to be about five months pregnant, and on looking up her history, it was found that she had been in the dairy pasture where the dairy bull was given exercise in December, 1921, prior to going into the experiment. She was, therefore, turned into the road pasture with the bred heifers of this group and Group I, although she had been vaccinated on February 7 when about two months pregnant.

On May 18, in the morning, the unbred heifers of Groups I and III in the east pasture got through an open gate into the road pasture with the bred heifers of the same groups. While the attendant was arranging gates in the corral where the animals had been placed with the bulls prior to separating them into bred and unbred groups, bull 412 was observed to breed no. 2318 and she was found to have a vaginal discharge. The laboratory was notified and, upon making a rectal examination, the uterus was found to be empty. The hand could be easily passed into the vagina, which contained a mucopurulent material streaked with blood, a handful of which was withdrawn and placed in a sterile tube. The cervix was open sufficiently to admit two fingers. On massaging the uterus per rectum with the other hand cupped over the cervix, some shreds of tissue with blood clots were expressed and placed in a second sterile tube. An effort was made to demonstrate *Bacterium abortum* by microscopic examination, but it was poor material to use for this purpose and the organism could not be demonstrated. Two guinea pigs, 2221 and 2222, were injected intra-abdominally with a salt solution suspension



of this material. Both were found to be normal when killed and examined August 2, 1922. This animal had definitely aborted between May 12, when she was found to be five months pregnant, and May 18, when she was seen to accept service from the bull. During this period the unbred heifers of this group and of Group I were being corralled twice daily with the bulls, but the bred heifers were not being closely watched. No evidence of the aborted fetus or membranes could be found in a careful search of the road pasture, which was to be expected owing to the fact that the area is hilly and covered with brush-growth in some places. Small predatory animals, including coyotes, infest the area. In examining this animal for pregnancy on May 12, a rectal examination only was made. This animal was continued in the experiment. She did not conceive from the service of May 18, but did so after the bulls were turned with this group in the far-east pasture on June 24, 1922. This pasture, in which this group was placed, had not contained any infected animals and they were left there for the remainder of the experiment, thus being kept free from any infection except that given them in the vaccination, February 7, 1922. At the time they were placed in the far-east pasture, no. 413 was the only one that was pregnant.

#### *History of First Pregnancy of Animals, Group III.*

These animals were examined for pregnancy on September 26, 1922, and nos. 413, 2297, 2318 and 2321 were the only four that were pregnant. Bull 411 was, therefore, left with this group and bull 412 was removed to Group IV-A in the east Rifle Range (figure 1). Later examinations for pregnancy on November 20 and December 29, 1922, and February 26, 1923, showed the above animals to be the only ones that had become pregnant.

On February 5, 1923, bull 411 was in poor condition and was removed from the far-east pasture and placed with Groups I and II which were corralled around the buildings (figure 1) so that he could be fed hay. He was replaced by bull 412, which had been with five heifers in Group IV-A and was in very good condition. On February 12, bull 412 jumped the fence from the far-east pasture to the east pasture where he bred cow no. 20, an infected control in Group II-A, which had aborted September 10, 1922. On that date both bulls were placed in a special corral built for them in the connecting pasture (fig. 1).



The animals in Group III had been in continuous association with one or both bulls from April 10, 1922, to February 12, 1923, a period of approximately ten months, and only four of them had become pregnant. The far-east pasture in which these animals were kept is large, rough and hilly, with considerable brush-growth on the hill-sides. They were not, therefore, under very close observation during all of this period. It could have been possible for them to have become pregnant and to have aborted without being observed. We do not feel, however, that this occurred because of the repeated negative examinations for pregnancy made during the period. It is also improbable that abortions occurred in any of the six animals before a diagnosis of pregnancy was made, and did not occur in any of the four animals in which pregnancy was early diagnosed. The results obtained with the animals in Group I further substantiate the improbability of any of those in Group III having aborted. All six of the animals finally became pregnant between May 1 and the end of September, 1923, and calved normally (see table 2). No abortion organisms were found in the colostrum or placentae of any of these animals. The interesting result in Groups I and III is that of the thirty vaccinated animals, only twenty-one were successfully impregnated within a reasonable time. Eight of the nine finally became pregnant. Six of the nine were constantly in association with one or both bulls for ten months and the other three for the period from April 10 to June 24, 1922. Little difficulty was experienced in getting the control animals of Group II bred. The nine in Groups I and III that failed to get with calf had never been pregnant and they were the youngest heifers.

The only difference in the treatment of the animals in Groups I and III from the controls in Group II was that the former received an injection of living *Bacterium abortum* organisms. At the time, it was concluded that this must have been responsible for the failure of these animals to get with calf. Later a somewhat similar experience was obtained in getting the five animals in Group IV-A with calf. Nothing was done to interfere with the breeding of the animals in Group IV-A, and this has caused us to look for other possible explanations.

It has long been recognized that feed conditions play an important role in the development of estrum. Animals on range have fre-

quently been known to have ovulation delayed for many months when feed was poor. Our experimental animals were kept under semi-range conditions and during the dry season the supply and quality of the feed was such that the animals were not kept in very good flesh, even though the natural feed was augmented by alfalfa or grain hay in small amounts.

The green feed conditions during the last two years have not been good on account of shortage of rainfall. The problem of nutrition, therefore, may have played a part in the failure of these animals to get with calf.

### *History of Second Pregnancy of Animals, Group III.*

In this group only four animals became pregnant the first year. Two of these four were killed shortly after calving and their body tissues examined for *Bacterium abortum*. The other two, nos. 2318 and 2321, conceived again promptly and carried their calves to term, thus terminating their second pregnancy at about the time the other six animals in this group were completing their first pregnancy. Guinea pig inoculation of placentae and colostrum from these animals were negative for *Bacterium abortum* (see table 2).

### EXPERIMENTAL DATA ON ANIMALS OF GROUP IV

The eleven animals in this group were assembled in August, 1922, approximately one year after those in the other groups. Group IV-A was placed in the east rifle range and Group IV-B in the rifle range (fig. 1). They were to be bred without any previous treatment to ascertain whether the male would transmit infection to them in the process of breeding.

On September 26, 1922, bull 412 was removed from Group III in the far-east pasture and placed with Group IV-A. From that time to February 5, 1923, he was constantly in the pasture with them, and thereafter they were seen daily for the presence of estrum although the bulls were kept corralled after February 12, 1923. Despite the fact that no previous treatment had been given them, these animals came in heat slowly. Two of them were bred in December, 1922, one on March 24 and again on May 21, 1923, one on May 27, 1923, and the fifth animal not until about August 1, 1923. A period of ten months was therefore required to get these animals with calf. They

all carried their calves to term (see table 2) and calved normally, except no. 437 which died during parturition from a ruptured uterus.

Opportunity was offered to breed only one of the six animals in Group IV-B by the bull after breeding an aborting animal, and this was some months after the abortion occurred. On April 7, 1923, bull 412 bred cow 429, Group II-A, which had aborted on September 7, 1922, and the same date, following this service, was bred to cow 444, Group IV-B, and she became pregnant to this service. This latter animal calved normally January 11, 1924, and the colostrum was free from *Bacterium abortum* organisms. The examination of the placenta was incomplete.

One other animal in this group, 442, was bred January 2, 1923, but at that time none of the aborting animals had been bred. She also calved normally on October 10, 1923.

The other four animals in Group IV-B were not utilized. They were removed January 31, 1924, and used in another experiment.

The animals which calved in Group IV, A and B, were killed and their body tissues examined for the presence of *Bacterium abortum*. The blood reactions of all of these animals remained negative. The result of the test of the blood sample from no. 445, Group IV-A, on July 11, 1923, was not checked until after the sample was discarded and, therefore, was not retested.

#### STUDY OF BODY TISSUES FOR PRESENCE OF BACTERIUM ABORTUM IN ANIMALS FROM ALL GROUPS THAT HAVE DIED OR BEEN KILLED

To date, twenty-four animals have died or been killed: six were from Group I, four from Group II, seven from Group III, and seven from Group IV. Cultures and guinea pig inoculations have been made from the following tissues with negative results for *Bacterium abortum* in every instance:

##### GROUP I.

No. 407. Died December 28, 1922. Atlantal, submaxillary, mediastinal, mesenteric, supramammary, internal iliac, precrural glands, udder, placenta and point of inoculation of vaccine.

No. 424. Killed February 28, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, gastric, mesenteric, renal, supramammary, internal iliac, precrural, precapular, cervical glands, udder, liver, spleen, uterus and cervix.

No. 421. Killed March 23, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, gastric, portal, mesenteric, supramammary, internal iliac, precrural, prescapular, cervical glands, liver, spleen, uterus and cervix.

No. 428. Killed February 21, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen, and uterus.

No. 405. Killed July 8, 1924. Supramammary glands, udder, uterus and placenta.

No. 25. Killed September 20, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen and placenta.

#### GROUP II.

No. 431. Died October 22, 1923. Supramammary glands and placenta.

No. 2060. Killed February 21, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen and uterus.

No. 26. Killed May 6, 1924. Supramammary glands, uterus and placenta.

No. 2312. Killed May 6, 1924. Supramammary glands, uterus and placenta

#### GROUP III.

No. 413. Killed May 21, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen, uterus, ovary and point of inoculation of vaccine.

No. 2297. Killed May 21, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen, ovary and uterus.

No. 2313. Killed February 21, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen and uterus.

No. 402. Killed May 13, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, spleen and uterus.

No. 419. Killed May 22, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, spleen and uterus.

No. 2321. Killed May 22, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, and spleen.

No. 406. Killed September 20, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen and uterus.

#### GROUP IV.

No. 437. Died September 26, 1923. Supramammary glands and placenta.

No. 435. Killed November 20, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, gastric, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, liver, spleen, ovary and uterus.



No. 442. Killed November 20, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, gastric, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, liver, spleen, ovary and uterus.

No. 444. Killed February 4, 1924. Supramammary glands and uterus.

No. 438. Killed May 13, 1924. Atlantal, submaxillary, retropharyngeal, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, spleen and uterus.

No. 445. Killed May 13, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, spleen and uterus.

No. 439. Killed May 22, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, spleen and uterus.

In order to conserve guinea pigs, which were very difficult to secure when some of the animals were killed, parts of from two to five lymph glands were ground with physiological salt solution and the resulting suspension injected into a single guinea pig. While the findings were all negative, it was, nevertheless, thought desirable to list each animal separately in order to give the reader the essential details of the examinations in each case. The following is the comment which we have to make in regard to the above data:

*Bacterium abortum* not having been found in the cultures, they were recorded as negative. A variety of bacteria was obtained in some of the cultures, but none had significance in the problem under study. One hundred and thirty-six guinea pigs were used. All survived the inoculation and were killed at the end of about six weeks except the four from cow 437, Group IV-A. This animal died during parturition from a ruptured uterus and the guinea pigs were inoculated with tissues from her supramammary glands and placenta. They died in forty-eight hours following the injection.

Cow 421, Group I, which was killed on March 23, 1923, showed *Bacterium abortum* in her colostrum at time of calving on March 6. When slaughtered, the supramammary lymph glands only were removed for examination as it was not known that the colostrum was positive until the guinea pigs inoculated with it were killed on April 25.

In studying the agglutination tests of the blood of these animals (table 1), it will be seen that nos. 407, Group I, and 2060, Group II-A, were the only ones giving a positive reaction at time of slaughter. The examination of the tissues of both of these animals



was rather extensive. Failure to find the organism is either evidence that our examination was not sufficiently searching or that some animals may give a positive agglutination test for weeks or months after they no longer harbor the live organism. In one cow under our observation free from *Bacterium abortum* infection given repeated injections of dead organisms, a positive agglutination test remained for a period of at least nine months. This case is discussed in the attached footnote.\*

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\* The research of Smith<sup>19</sup> and his associates at the Rockefeller Institute of Animal Pathology has demonstrated that *Bacterium abortum* agglutinins do not pass the placental filter. It has also been shown by others that other immune bodies do not pass through the placenta of bovines and some other ruminants. Nevertheless, it is true that in some other species of animals such passage has been demonstrated. It occurred to us that it would be desirable to eliminate the remote possibility of the absorption of agglutinins by *Bacterium abortum* in the fetus and its membranes by demonstrating the failure of their passage in a non-infected, gravid uterus. We, therefore, developed large quantities of these substances in the blood of a cow just prior to parturition without the uterus being infected so that any agglutinins which passed the filter would be demonstrated in the blood of the fetus at birth.

With this object in view, a suspension of *Bacterium abortum* organisms was made. These were killed by heating to a temperature of 60° C. for fifty-eight minutes and raising the temperature to 65° C. for two minutes. After cooling, three glycerine agar tubes were heavily inoculated with the suspension by means of a sterile glass pipette, and no growth was obtained. To further test the sterility of the suspension, guinea pig 3485 was inoculated with 1 mil of the material on November 27, 1923. This guinea pig was killed on January 8, 1924, and found to be free from infection.

One of our dairy cows, no. 2451, in advanced pregnancy, free from *Bacterium abortum* infection and with negative blood reaction, was injected subcutaneously on the left side of the neck with 5 mils of this suspension of dead *Bacterium abortum* organisms on December 3, 1923. Blood taken from the animal on December 8 was still negative. On December 10, 10 mils of the same suspension were injected on the opposite side of the neck. On December 13, blood from this animal gave a positive agglutination test up to 1-100 dilution. On December 17, 5 mils of the same suspension were injected into each of three different places on the right side of the neck. On December 22, blood from this cow gave a complete agglutination in dilutions up to 1-500 and a partial agglutination in a dilution of 1-1000. She calved normally on December 23 at 10 A.M. The calf was prevented from getting colostrum and a sample of its blood was taken as well as one from the dam. One quart of the colostrum was collected from the udder in approximately equal amounts from all four teats. An agglutination test of the colostrum serum was positive in 1-1000 dilution and of the blood serum a partial agglutination was obtained in this dilution, the same as with the sample taken on the previous day. The agglutinins had, therefore, become more concentrated in the colostrum than in the blood. The serum of the calf was completely negative in all dilutions from 1-25 up.

After the samples were taken from the dam and the calf, the latter was allowed to suckle on the evening of December 23, 1923. On the following morning, blood was again drawn from the calf and gave complete agglutination in dilutions up to 1-1000. Thereafter the agglutinin content of the blood of the cow remained sufficiently high to produce complete agglutination in dilutions over 1-100 for at least nine months. Smith and Little<sup>18</sup> found a persistence of agglutinins produced by the injection of heat-killed organisms in presumably uninfected but exposed heifers for a period of 6½ to 7 months.

## ELIMINATION OF BACTERIUM ABORTUM IN MILK OF COWS VACCINATED WHILE IN LACTATION

In the series of experiments which has already been discussed, all of the animals that were vaccinated received the injection when they were not in lactation. Opportunity was therefore not afforded to make a study of their milk until the following parturition, almost a year after the live abortion organisms had been administered. At this time it was found that only four of the vaccinated animals eliminated the organisms in their milk and all of them were in Group I which received heavy infection orally in addition to the vaccination.

In order to get information on the question of the elimination of the organisms in the milk of cows that were lactating at the time of vaccination and other questions of importance in the study of the immunity and carrier problem, a second series of experiments was started in December, 1923, with the animals in the University Dairy herd. These animals constituted a particularly valuable group for this purpose because they were known to have been free from *Bacterium abortum* infection for a period of over three years.

Schermer and Ehrlich<sup>17</sup> in an effort to answer the above question inoculated subcutaneously each of three cows with the growth of a slant agar culture of *Bacterium abortum* and at the end of one, two, three and five weeks respectively, milk was taken from each of the animals. It was centrifuged and cultured and in no case did they recover the organism. They also inoculated subcutaneously one cow with five agar tube cultures showing good growth and at the end of seven and thirteen days respectively, milk samples were collected and injected into guinea pigs. Blood samples were taken from the guinea pigs periodically and always showed, according to the authors' statement, a titer under 1-100. The guinea pigs were killed nine weeks after the inoculation and showed no lesions of abortion. Cultures made from their organs were free from *Bacterium abortum*.

Zeller<sup>22</sup> also concerned himself with this question and performed the following experiments. One cow was inoculated subcutaneously behind the shoulder with 10 slant agar cultures in 20 mls of salt solution. On seventeen occasions, from one to eighty-nine days after the inoculation, samples of blood, milk, saliva, feces and urine were cultured for the presence of *Bacterium abortum* with negative results

in all cases. The animal was killed 128 days after the injection, and cultures from the internal organs gave negative results. Inoculation of four guinea pigs with the spleen and udder also resulted negatively.

Another cow was inoculated with 20 slant agar cultures in 30 mils of salt solution. On sixteen occasions, from three to sixty-seven days subsequently, the same materials as those taken from the previous cow were cultured with negative results. On each of these occasions one guinea pig was inoculated with cream and sediment of milk from all four quarters. At the end of two months the guinea pigs were killed and found free from infection. Extensive cultures from their body tissues were negative and their blood gave a negative agglutination test.

A third cow was inoculated as above and on twenty-one occasions, from one to sixty-five days subsequently, milk, blood, etc., were cultured and one guinea pig inoculated with the milk. All cultures and guinea pigs remained free from *Bacterium abortum* infection.

A fourth cow, not in lactation, was inoculated with 10 agar slant cultures. At various intervals samples of all the previously mentioned materials except milk were collected from the animal and inoculated into cultures and guinea pigs with negative results. At 161 days after the injection the animal was slaughtered and her tissues cultured with negative results. Guinea pigs inoculated with the material from the spleen, uterus, ovary, udder and supramammary lymph glands were negative.

In our own investigations of this question during the past year sixteen head of non-pregnant milking cows have been vaccinated. The live abortion organisms used in this vaccine were the same as those used in the first series of experiments except that strain 101 was replaced by strain 150, which had been more recently isolated (September 25, 1922) from an aborted bovine fetus. These organisms were grown on solid media only. The quantity of organisms injected was one-half the number used in the first series or less.

The material was prepared by having the opacity of the suspension equal to a two billion meningococci standard in place of a four billion as used in the first series of experiments. This showed a Gates<sup>7</sup> reading of 1.5 cm. With the plate culture method of counting, this suspension was found to contain from 4.47 to 6.8 billion organisms per mil. Hagan<sup>9</sup> estimated by the plate method that a

*Bacterium abortum* suspension of 2.4 cm. Gates' reading varied between 4.5 and 13 billion organisms per mil. When we diluted our suspension of 1.5 cm. Gates' reading to equal 2.4 cm. and used Hagan's average of 8.74 billion per mil, we found our suspension of 1.5 cm. was closer to his lower count.

To make 20 mls of this concentration required about one slant agar culture of the organism.

These various methods of estimating the number of organisms are given in order that our dose may be compared with that used by other workers. The number of mls of the suspension given to each cow varied and is given in table 5.

The vaccine was administered subcutaneously at several places and in some cases additional dilutions were made to obviate local swelling and abscess formation as far as possible. Great care was used in vaccinating the animals to prevent any contamination of the premises. In four of the cases local swellings developed into abscesses. These animals were isolated during the surgical removal and healing of the abscessed areas. Eighteen controls have been in association with the vaccinated animals and that no transmission of the infection has so far taken place has been determined by regular guinea pig inoculations with milk samples and monthly agglutination tests of blood samples.

After the vaccination, samples of milk were collected, in some cases daily for three days and in all cases weekly for two or three weeks. Samples of milk from all the animals in the dairy barn were tested for *Bacterium abortum* in a routine manner at intervals of one to two months.

The samples of milk were collected, before the regular milking, in sterile, wide-mouthed bottles, about one-fourth of the sample being taken from each quarter, with a total of 300 to 400 mls. In some cases a similar sample was collected after the milking machine had been removed and before stripping was started. No particular difference in the *Bacterium abortum* content of the two kinds of samples has been found.

Ten of the sixteen lactating animals that were vaccinated have given off *Bacterium abortum* in their milk. In no case has this organism been definitely isolated during the first three days after vaccination. From the end of this period to the seventh day, no samples



TABLE 4.—TEMPERATURE REACTIONS OF VACCINATED ANIMALS IN SECOND SERIES OF EXPERIMENTS WITH RESULTS OF MILK EXAMINATIONS FOR THE PRESENCE OF BACTERIUM ABORTUM

No. of animal	Date of vaccination	Tem- perature when vac- cinated	Amount of vaccine	Temperature for five days subse- quent to vaccination					Weekly milk examinations following vaccination								
				106.2	105.8	104.6	101.8	102.8	Date	Number of guinea pigs inoc- ulated	Result	Date	Num- ber of guinea pigs inoc- ulated	Result	Date	Num- ber of guinea pigs inoc- ulated	Result
2399	Dec. 18, 1923	102.6	10 mls	106.2	105.8	104.6	101.8	102.8	Dec. 24	3519	Both	Dec. 31	3529	Both	.....	.....	.....
2400	Dec. 18, 1923	102.2	10 mls	106.0	106.2	105.4	105.4	104.0	Dec. 24	3520	Positive	Dec. 31	3530	Positive	.....	.....	.....
2401	Dec. 18, 1923	102.0	10 mls	104.5	106.0	104.4	105.1	104.2	Dec. 24	3521	Both	Dec. 31	3531	Both	.....	.....	.....
2421	Dec. 18, 1923	103.0	10 mls	106.0	107.4	105.4	105.0	103.8	Dec. 24	3522	Negative	Dec. 31	3532	Negative	.....	.....	.....
2154	Jan. 5, 1924	101.6	10 mls	106.7	106.0	103.8	103.8	101.6	Jan. 14	3523	Both	Dec. 31	3533	Both	.....	.....	.....
									Dec. 24	3524	Negative	Dec. 31	3534	Negative	.....	.....	.....
									Dec. 24	3525	Both	Dec. 31	3535	Both	.....	.....	.....
									Jan. 14	3526	Negative	Jan. 23	3536	Negative	.....	.....	.....
										3547	Both	Jan. 24	3549	3550 died	.....	.....	.....
										3548	Negative		3550	3549 posi- tive	.....	.....	.....
440	Jan. 31, 1924	101.4	20 mls	106.8	106.2	104.2	102.2	103.2	Heifers	not milki ng.							
441	Jan. 31, 1924	101.6	20 mls	106.6	105.8	103.8	105.0	104.0									
2055	Mar. 8, 1924	101.2	15 mls	106.8	106.6	105.0	104.6	105.0	Mar. 14	Milk		Mar. 21	3754	Positive	Mar. 28	3762	Positive
2142	Mar. 8, 1924	101.4	15 mls	106.6	105.8	104.8	103.2	104.8	Mar. 14	samples		Mar. 21	3755	Negative	Mar. 28	3763	Positive
2405	Mar. 8, 1924	101.2	15 mls	106.4	106.0	105.2	103.6	103.2	Mar. 14	Cultured		Mar. 21	3756	Negative	Mar. 28	3764	Positive
2143	Apr. 14, 1924	102.4	10 mls	103.2	106.8	104.0	102.2	102.4	Apr. 22	Negative		Mar. 21			May 5	3820	Positive
2504	Apr. 14, 1924	102.0	10 mls	106.0	105.8	105.0	103.8	102.6	Heifer	not milk- ing.							
2144	May 19, 1924	102.6	10 mls	106.0	106.0	105.8	103.8	.....	May 28	3896	Negative	June 2	3899	Negative	June 9	3903	Negative
1441	May 19, 1924	102.4	10 mls	105.6	107.0	106.0	102.6	.....	May 28	3895	Negative	June 2	3898	Positive	June 9	3904	Negative
2062	July 7, 1924	102.4	10 mls	105.6	106.4	105.4	103.2	.....	July 14	3933	Negative	July 22	3979	Negative	July 28	3901	Negative
2316	July 7, 1924	102.6	10 mls	107.0	106.2	105.2	105.6	.....	July 14	3934	Negative	July 22	3980	Negative	July 28	3902	Negative
									July 14	3951	Negative	July 22	3980	Negative	July 28	3981	Negative
2304	Aug. 25, 1924	101.6	10 mls	106.4	106.6	104.8	103.2	101.2	Sept. 1	3952	Negative	Sept. 10	4047	Negative	Sept. 15	4048	Positive
1438	Oct. 14, 1924	101.8	10 mls	105.6	105.2	104.6	103.6	104.3	Oct. 20	4129	Negative	Oct. 28	4133	Negative	Nov. 4	4141	Negative
2503	Oct. 14, 1924	102.4	10 mls	105.8	106.0	104.7	102.2	102.2	Oct. 20	4130	Negative	Oct. 28	4131	Negative	Nov. 4	4142	Negative



TABLE 4.—TEMPERATURE REACTIONS OF VACCINATED ANIMALS IN SECOND SERIES OF EXPERIMENTS WITH RESULTS OF MILK EXAMINATIONS FOR THE PRESENCE OF BACTERIUM ABORTUM—(Concluded)

Monthly to bimonthly examinations of milk following vaccination											
No. of animal	Date of first sample	Num-ber of guinea pigs inoc-ulated	Result	Date of second sample	Num-ber of guinea pigs inoc-ulated	Result	Date of third sample	Num-ber of guinea pigs inoc-ulated	Result	Date of fourth sample	Num-ber of guinea pigs inoc-ulated
2399	Feb. 14	3628	Both Positive	Apr. 14	3789	Positive	May 28	Dry	.....	July 14	Dry
2400	Feb. 14	3629	Negative	Apr. 14	3783	Negative	May 28	3881	Negative	July 14	3953
2401	Feb. 14	3616	Negative	Apr. 14	3788	Negative	May 28	3885	Negative	July 14	3954
2421	Feb. 14	3627	Negative	Apr. 14	3791	Negative	May 28	3886	Negative	July 14	3955
2154	Feb. 14	3632	Both Positive	Apr. 14	3780	Positive	May 28	3880	Positive	July 14	3956
440	.....	3633	.....	.....	.....	.....	.....	.....	.....	.....	3960
441	.....	3608	.....	.....	.....	.....	.....	.....	.....	.....	3943
2055	.....	3609	.....	.....	.....	.....	.....	.....	.....	.....	3944
2142	.....	.....	.....	Apr. 14	3796	Positive	May 28	3891	Positive	July 14	3931
2405	.....	.....	.....	Apr. 14	3799	Positive	May 28	3894	Negative	July 14	3932
2143	.....	.....	.....	Apr. 14	3798	Positive	May 28	3893	Negative	July 14	3937
2504	.....	.....	.....	Apr. 14	3795	Negative	May 28	3890	Negative	July 14	3938
2144	.....	.....	.....	.....	.....	.....	May 28	3896	Negative	July 14	3938
1441	.....	.....	.....	.....	.....	.....	May 28	3895	Negative	July 14	3940
2062	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3941
2316	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3942
2304	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3942
1438	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3941
2503	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3942

NOTE.—Each time the five monthly to bimonthly samples were taken, milk from every cow was tested. The unvaccinated animals numbering from eleven to twenty were always negative for Bacterium abortum.

were taken. In one animal the milk was definitely positive at the end of the first week. The milk of the remaining nine was demonstrated to be positive from the thirteenth to the fifty-fifth day.

The data on these milk examinations so far as it has been carried at the present time are given in table 4.

### DISCUSSION

Throughout the presentation of the paper, comment on the experimental data is made. The detailed work involved in the study of the problem under consideration is difficult to present in a manner to be easily followed. To obviate this, we have placed the data as far as possible in tables. On account of the care used in collecting and examining the experiment animals and the keeping of the various groups in pastures so situated that infection had no opportunity to spread from group to group, we have given the exact procedures that were carried out throughout the work for those who desire to study or criticize them.

The data show that of the twenty animals in Group I, seventeen became pregnant in from 70 to 137 days after the vaccination. From two to sixty-nine days after their impregnation these animals were given severe infection orally. All of them, except no. 407, which was accidentally killed late in gestation, dropped calves at the termination of a normal gestation period, ranging in these particular cows from 271 to 292 days. In one animal, no. 2305, the calf was born dead at the end of a 278-day gestation period. The placentae in all of these cases were free from *Bacterium abortum* infection.

In direct association with these animals were the ten controls of Group II-A, which were infected but not previously vaccinated. The infection took place at the same time and in the same manner as that given to the animals of Group I. Six of the animals in Group II aborted fifty-six to eighty-six days after the infection given orally. The placenta in every one of these cases showed the presence of *Bacterium abortum*. In addition this organism was found in the placenta of one of the remaining four animals in this group which calved twenty-seven days after infection was given. In regard to the remaining three animals, one calved ten days after the infection was given and the other two 160 days afterwards. The placentae of all three were free from *Bacterium abortum* infection.

These results justify the statement that the subcutaneous injection of living *Bacterium abortum* in the animals of Group I protected their fetuses and membranes from the same exposure to infection that caused the infection of seven out of ten animals in Group II. The animals of Group I were not so far advanced in pregnancy as those of Group II when the infection was given orally. However, in addition to the original infection, they were exposed to the aborted fetuses and discharges from the animals of Group II that aborted between August 21 and September 20, 1922. This gave them ample opportunity to pick up further infection when they were in the middle of their gestation periods. The thirty-four animals constituting all of Groups I and II, except no. 436, were penned every night in a corral of about one-half acre, and fed hay placed on the ground. Four of the abortions took place here. Further evidence that this was a natural exposure is demonstrated by the fact that all four of the association animals of Group II-B, which were with these animals while the abortions were taking place, picked up the infection although none of them aborted. (See table 2.)

Incidentally, the experiments indicate that abortion infection and premature expulsion of the fetus can be induced by a single exposure to infectious material given orally. The material used for infection consisted of fetal tissues, milk and cultures of *Bacterium abortum*. This was because we desired to give the animals a very severe exposure and to include material from sources that would constitute as nearly as possible the usual means of spreading the organism from the carrier of the infection to the healthy animal. In the mixture there were, of course, organisms other than *Bacterium abortum* present, but the latter were without question responsible for the abortions obtained since the animals of Group I were protected against *Bacterium abortum* only and did not abort.

In the matter of the ability of cultures of *Bacterium abortum* to produce abortion, it appears appropriate at this time to call attention to the fact that we have since our experiments began vaccinated three pregnant animals with live *Bacterium abortum*, although the presence of pregnancy was not known at the time the vaccine was given. All of these animals aborted and in two of the cases where the fetus and membranes were available for study, *Bacterium abortum* was readily recovered. One of these animals, no. 2318, Group III, previously

mentioned, aborted a five months fetus a little over three months after the vaccination. The other two were in the second series of experiments. These animals were heifers and were not examined for pregnancy prior to vaccination as the foreman had no record of their having been bred. They were nos. 2420 and 2424 and were both vaccinated December 18, 1923, when about four months pregnant. No. 2420 aborted March 5 and No. 2424 on March 12, 1924, seventy-eight and eighty-five days respectively after the vaccination.

In a previous publication<sup>10</sup> reporting the progress of these experiments, it was stated, "Nine of the 30 vaccinated animals have up to date (May, 1923) failed to conceive." Since that date, eight of the nine head have conceived and calved normally between February 8 and June 26, 1924. It was at that time also concluded that "no other explanation can be offered for this sterility except the vaccination." In the fall of 1922 we extended our experiments to include the animals of Group IV, an untreated and non-infected group. The five animals in Group IV-A required a period of ten months for all of them to conceive. The effect of poor feed conditions entered here as a factor. While at this time we cannot entirely absolve the vaccination from being a causative factor in the failure of the nine animals of Groups I and III mentioned above to conceive, we should not fail to consider that other factors, particularly feed conditions, may have shared in the cause of the temporary sterility.

The blood tests of this fairly large group of animals taken monthly over a period of nearly three years with known time and method of infection and also certain definitely ascertained dates of elimination of *Bacterium abortum* from some of the animals furnishes data deserving comment.

It will be observed from table 1 that the thirty animals in Groups I and III were infected subcutaneously on February 7, 1922, before which time all had been negative to the agglutination test. Fourteen days later all of the animals were definitely positive. On June 26, 1922, the ten animals of Group II-A, all negative to the blood test, were infected orally. Fourteen days later all of the animals, with the possible exception of no. 401, were definitely positive. No blood samples were taken earlier than fourteen days after exposure in these cases. In some other experimental work where five animals were infected orally with cultures of *Bacterium abortum*, two showed



definite reactions at the end of eight days and all at the end of fourteen days. In the vaccination of the animals in the second series of experiments, positive agglutination tests were obtained at the expiration of seven days in several cases. Zeller<sup>22</sup> reports positive agglutination tests in a titer of 1-100, six days after subcutaneous injection. Smith and Little<sup>18</sup> demonstrated positive agglutinations in vaccinated and presumably uninfected, exposed cattle ten days after vaccination. With our cultures it appeared that oral administration was just as effective in producing a positive agglutination titer in the dilutions used as subcutaneous inoculation and that the time required was regularly within fourteen days.

In studying the persistence of the agglutination reaction in animals after a single exposure to infection by subcutaneous inoculation, when non-pregnant, Group III shows that while some of the animals after a period of a few months became negative, others persisted for a longer time even up to one year. The presence or absence of pregnancy intervening during this period seemed to have little effect on the agglutination titer. In comparing the agglutination reactions of the animals of this group with those of Group I which were vaccinated and infected, it will be observed that in a general way they were practically the same with the notable exception of the two animals, nos. 4 and 408 of Group I, which remained eliminators of the organism at the expiration of their second pregnancy. On the other hand, there was a persistence of the agglutination titer in the non-vaccinated but infected animals of Group II-A which continued to harbor and eliminate the organism at the end of the second pregnancy.

In correlating the agglutination titer and the elimination of *Bacterium abortum*, we have attempted to see whether animals discharging these organisms always have a titer of at least 1-100 as strongly suggested by Schroeder and Cotton<sup>16</sup> in their studies of the milk from fifty-six cows of which the blood serum of thirty reacted in dilutions of 1-200 or higher and twenty-six showed an agglutination titer of less than 1-100. Twenty-five of the thirty high reacting cows were eliminating *Bacterium abortum*, while none of the twenty-six low reacting cows were doing so. In work along this line on the University Farm herd at Davis by Hayes and Barger,<sup>11</sup> it was found that the herd contained fifteen reactors to the agglutination test in



TABLE 5.—SHOWING REGULAR MONTHLY AGGLUTINATION TESTS NEAREST TO THE DEFINITE DATES WHEN BACTERIUM ABORTUM WAS KNOWN TO HAVE BEEN ELIMINATED FROM ANIMALS OF GROUPS I AND II

Group Number	No. of Animal	Calved or aborted	Agglutination test		Positive milk samples	Agglutination test Nov. 10, 1922	Second calving date	Agglutination test	
I	4	Jan. 23, 1923.....	Jan. 10	Feb. 14			Feb. 22, 1924.....	Jan. 30	Feb. 27
	408	Jan. 25, 1923.....	± ± ± +	± ± ± ±				± ± ± ±	± ± ± ±
	421	Mar. 6, 1923.....	± ± ± -	± ± - -			Feb. 23, 1924.....	± ± ± +	± ± ± ±
	426	Mar. 24, 1923.....	Feb. 14	Mar. 23					
			± - - -	± - - -			Mar. 19, 1924.....	Feb. 27	Mar. 25
			Mar. 23	Apr. 17				± - - -	± - - -
			± + ± -	± - - -					
II	401	July 23, 1922.....	July 10	Aug. 10			Feb. 28, 1924.....	± ± ± +	± ± ± +
	2181	Aug. 21, 1922.....	± ± - -	± ± ± ±				Jan. 30	Feb. 27
	2180	Sept. 2, 1922.....	Aug. 10	Sept. 12	Nov. 10, 1922	+ + + ±	Feb. 19, 1924.....	± ± ± +	± ± ± ±
	429	Sept. 7, 1922.....	± ± ± -	± ± ± ±	Nov. 10, 1922	+ ± ± ±	Apr. 27, 1924.....	± ± ± ±	± ± ± -
	416	Sept. 7, 1922.....	± ± ± +	± ± ± ±	Nov. 10, 1922	± ± ± ±	Jan. 3, 1924.....	Dec. 27	Jan. 30
	20	Sept. 10, 1922.....	± ± ± ±	± ± ± ±	Nov. 10, 1922	- ± - ±	Nov. 13, 1923.....	± ± ± -	± ± ± -
	431	Sept. 20, 1922.....	± ± ± ±	± ± ± ±	Nov. 10, 1922	± ± ± ±	Nov. 20, 1923.....	± ± ± +	± ± ± +
	183	Feb. 16, 1923.....	Feb. 14	Mar. 23				Oct. 30	Nov. 27
	430	Apr. 27, 1923.....	± - - -	± - - -				± ± ± +	± ± ± +
	2298	Feb. 3, 1923.....	Apr. 17	Mar. 12				± ± ± -	± ± ± -
	2317	Mar. 16, 1923.....	± - - -	± - - -				± ± ± -	± ± ± -
			Jan. 10	Feb. 14				Jan. 30	Feb. 27
			Feb. 14	Mar. 23				± ± ± +	± ± ± +
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dilutions of 1-100 or greater and seven of them were discharging *Bacterium abortum* in their milk. There was, however, one animal with a negative agglutination test that eliminated the organism in the milk. Fitch and Lubbehusen<sup>6</sup> found that fourteen of forty-eight animals that reacted to the agglutination test eliminated *Bacterium abortum* in the milk. The blood serum of these fourteen always had an agglutination titer as high as 1-100 at the time the milk specimen was received. We find that the data in tables 2 and 3 show that there have been twenty-nine occasions in which *Bacterium abortum* has been demonstrated to be eliminated from the bodies of fifteen of the animals in Groups I and II. At twenty-one of these periods, the agglutination titer was positive, at two it was very suspicious and at the remaining six it was practically negative.

The principal failures in this respect occurred in three of the association animals of Group II-B. However, none of these three animals remained permanent carriers. One animal in this group, no. 2298, was negative at the time of the first elimination of *Bacterium abortum* but remained a carrier and soon became definitely positive to the blood test and continued so to the end of the second pregnancy when she again eliminated the organism. The details of these observations we have prepared in table 5.

It has been demonstrated in these experiments and from other observations we have made that *Bacterium abortum* is eliminated from the udder or genital tract of cows when their agglutinating titer is under 1-100 or even entirely negative. It is well to recognize this limitation of the agglutination test in efforts to use it in controlling the disease. Nevertheless, in view of the fact that the eradication of the disease by the blood test method is recommended only in herds where the percentage of reactors is small, the law of proportion tends to reduce the importance of this limiting factor to a great extent.

In regard to the study of the body tissues of animals that have been killed in the experiments, we have nothing to add here to the discussion given in the text of the article.

It will be observed that although the fetuses and membranes of the animals in Group I were protected from infection, the organisms remained alive in the udder and were eliminated with the colostrum of four, or 25 per cent, of the animals that completed their gestation periods. Three of these four animals completed a second gestation

and in two of them it was again demonstrated in the colostrum at this time even though the membranes remained again uninfected and the fetuses were born at term and normal.

All of the animals in the first series of experiments were specially collected for the experiment and none of them were in lactation at the time of the vaccination. They did not offer an opportunity, therefore, to study the mammary secretion for the presence of the organism immediately after vaccination. This was afforded, however, when the second series of experiments was started to include the milking animals in the University Dairy. Immediately after the vaccination of these animals was under way and a study of their milk made, no difficulty was experienced in getting positive results. There was a total of sixteen lactating animals vaccinated and the milk of ten of these has up to date become positive in from seven to fifty-five days subsequent to vaccination. This has been contrary to the experience of other investigators of this disease, notably Schermer and Ehrlich and Zeller, whose work has already been reviewed. Its repeated occurrence in our animals following several different vaccinations leaves no doubt of the probability of *Bacterium abortum* organisms injected under the skin of the neck in lactating animals in the form of vaccine being discharged from the udder with the milk secretion in from one to several weeks after the vaccination. Whether they will remain permanently located in the udder in such cows, we have not had time to ascertain, but reference to table 4 will show that the organisms will continue in this location for a considerable time and to the end of the lactation period in which the animals were vaccinated. This further emphasizes the fact generally accepted that *Bacterium abortum* vaccine should never be used in uninfected herds—also, that there is danger in bringing recently vaccinated animals into abortion-free herds.

It will be observed in the first series of experiments that a marked reaction followed the administration of the vaccine to the animals of Groups I and III. No temperatures were taken of these animals. In the second series of experiments, temperatures were taken in all cases for several days immediately following the vaccination, and it was found that invariably there was a decided pyrexia. The temperatures are given in table 4. Similar observations were made by Smith and Little<sup>18</sup> and Schermer and Ehrlich.<sup>17</sup> In addition to the

temperature reaction, there was a temporary loss of appetite and reduction in the milk flow.

In the article reference was made to the abscess formation following the vaccination and the isolation of *Bacterium abortum* in the two cases which were studied. In the second series of experiments, we had abscess formation in four cases and in all of them the organism was readily recovered; in three of the animals, it was obtained in pure culture.

Many months were required to get the animals of Groups III and IV-A bred. We did not intend to breed the animals of Groups I and II the second time until all the animals of Groups III and IV-A were bred. Such a long period was required to complete the breeding of the latter groups, that it was necessary to proceed with the second breeding of the first two groups. The bulls were therefore moved back and forth to some extent among all four groups during 1923 and no ill effects were observed from this. The entire series of experiments failed to yield any evidence that the bulls transmitted infection mechanically in the process of breeding or otherwise. However, it must be recognized that good opportunity for the bulls to do so was probably not available. It will be observed in table 2 that the vaccination of Groups I and III occurred on February 7, 1922. The bulls bred the animals of Group I from April 10 to June 24. They were then placed with the animals of Group III. It was not until September 26, nearly seven months after the vaccination, that one of the bulls was placed with the animals of Group IV-A.

Attention is directed to the agglutination tests of bull 411. This bull and bull 412 were placed with the vaccinated Groups I and III on April 10, 1922. While the abscesses on these animals were healed at that time, some lurking infection from the discharges from these areas might have been present in the corral or pasture. The first blood test after that date was made on June 14, 1922, when bull 411 showed a positive agglutination in a dilution of 1-50 and continued to do so at irregular intervals for a considerable period of time afterwards. An agglutination at this dilution is generally considered below the positive point, especially when repeated tests fail to show an increase in the agglutination titer. These may be the normal agglutinins of this animal's blood or there is a possibility of this animal having picked up some infection from the vaccinated animals which he managed to keep in abeyance.

## CONCLUSIONS

1. The value of living cultures of *Bacterium abortum* in preventing abortion in the vaccinated animals when subjected to the identical infection that produced abortion in the control animals was demonstrated.

2. A correlation of the agglutination tests of the animals with the definite periods when *Bacterium abortum* was known to have been eliminated shows that this organism may be discharged from the body without its presence being suspected from the agglutination titer of the blood. This calls attention to the limitations of the agglutination test rather than demonstrates its inapplicability as a means to be used in the control of the disease.

3. It has been demonstrated that in a certain percentage of lactating animals injected with *Bacterium abortum* under the skin of the neck, the organisms so injected, or their progeny, will gain access to the udder and be eliminated with the milk. Vaccinated animals may, therefore, become spreaders of the infectious agent under these conditions and cannot with safety be moved into uninfected herds.

4. Animals that develop sufficient immunity to *Bacterium abortum* infection after vaccination to prevent abortion or disease of the placental tissues may still harbor the living organism and eliminate it from the udder.

5. Non-pregnant animals injected with living *Bacterium abortum* subcutaneously when not in lactation and not exposed to further infection failed to show the presence of the organism in the placenta or colostrum at the termination of the following pregnancy.

6. Animals exposed to *Bacterium abortum* in no other way except by vaccination will continue to give positive agglutination tests in a titer of 1-100 for several months to one year after the injection.

7. Vaccination of virgin heifers may be a factor in retarding their impregnation but this has not been satisfactorily demonstrated.

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A STUDY OF THE CONDUCTIVE TISSUES  
IN SHOOTS OF THE BARTLETT PEAR AND  
THE RELATIONSHIP OF FOOD MOVEMENT  
TO DOMINANCE OF THE APICAL BUDS

BY

FRANK E. GARDNER

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## NOTICE

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FRANK E. GARDNER†

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## INTRODUCTION

The tendency of most trees to produce branches from the terminal and apical buds, while the more basal buds of the shoot remain dormant, has long been a subject for observation and conjecture. This dominance of the upper buds tends to produce an excurrent type of growth which is striking in a number of fruit trees, particularly in many varieties of the sweet cherry and pear.

It is well known that ringing, wounding, bending, and other practices bring about the growth of buds which normally would remain dormant, though the reason for this response is not known. In the carbohydrate-nitrogen concept, a new aspect of growth has been evolved which may, when more clearly understood, furnish a basis for the treatment of trees in order to bring them into the proper balance of growth and fruitfulness. It is, then, from the standpoint of nutrition, that this study of dominance is undertaken.

Many explanations of the dominance of the apical buds have been advanced; among them Loeb's<sup>15</sup> inhibitor theory which is also sponsored by Reed and Halma.<sup>22</sup> This theory is not free from objectionable features and, moreover, seems to be as incapable of proof as of refutation.

Barker and Lees,<sup>3</sup> while favoring the inhibitor theory, feel that other factors, such as bud strength and sap supply to the buds, play an important rôle.

McCallum,<sup>18</sup> after studying what he considered to be the possible causes of dominance, concluded that "physiological correlation" is the factor responsible and that its effect probably acts along protoplasmic connections.

Child and Belamy<sup>5</sup> carry this conception still further by stating that the difference between the tip and the base of a shoot is a difference in the rate of fundamental metabolic reaction, such differences being associated with differences in protoplasmic condition and appearing in the form of gradients from the tip to the base. To these differences they apply the term, "physiological gradients."

In order to support their theory that dominance is not a matter of nutrition, these writers distinguish between two aspects of the relation of parts. The first is concerned with the conditions which permit or prevent the initiation of growth in a subordinate part, and the second,

with the amount of growth which may occur after its initiation. They believe that nutritive factors play a large part in the amount of growth, but that there are no reasons for believing that such factors initiate growth.

It is recognized that plants in order to grow must utilize both carbohydrate and nitrogenous foods. That these foods are moved from place to place within the plant is certain, yet there is no conclusive evidence as to what tissues are involved in the movement. However, it may be assumed that elaborated materials are translocated through one or perhaps both of the so-called "conducting elements"; namely, the sieve-tubes or the tracheal tubes.

Atkins<sup>1</sup> view is the usual conception of food movement in plants and is typical of that held by most physiologists. He states that the xylem tissue is essential for upward translocation of foods, but that downward movement of elaborated material is through the phloem and thence, by means of the medullary rays, into the inner portions of the tree for storage.

Curtis<sup>6</sup> departs from the orthodox position on this matter by stating that his experiments with ringing and defoliation show that carbohydrates, at least, move exclusively in the phloem.

Although Curtis conclusively showed that ringing interrupts the upward movement of carbohydrates, and Mason<sup>17</sup> found that downward movement is also checked in this way, it yet does not necessarily follow that translocation of these foods is restricted to the phloem. Dixon<sup>9</sup> points out that this interpretation rests on the fallacy of supposing that the removal of a ring of bark leaves the outer layers of xylem uninjured. It is the outer wood which he believes to be functional in food conduction, whereas the whole cross-section of xylem is available for water transport. It is his opinion that carbohydrates not only move upward through the xylem but that they also descend through this tissue.

Although the question of what tissues are involved in food conduction may at first appear unrelated to the matter of dominance of the apical buds, there is a possibility that buds develop into lateral branches as a result of food movement and its accumulation in some particular tissue.

In this study new evidence was sought which might lead to a better understanding of the nature of dominance in shoots and of what tissues are concerned in the conduction of foods.

## METHODS

*Influencing Nutritive Conditions.*—In order to influence the nutritive conditions of pear shoots and to cause buds to develop in lateral branches, several methods were employed:

1. Early in July and in succeeding months, shoots of the current season's growth were bent over through an angle of at least ninety degrees and tied in that position.

2. Portions of shoots were defoliated at intervals throughout the summer. In some cases the leaves were removed from the terminal half of the shoot, and in other cases from the basal half.

3. Shoots were ringed, and in some cases also defoliated either above or below the ring.

4. Shoots were headed-back on July 20 and 31.

It was planned to correlate the growth responses of the foregoing treatments with the carbohydrate and nitrogen contents of the shoots, but this phase of the problem was rendered unnecessary by the appearance of a contribution by Harvey<sup>12</sup> which contained complete tables of the analyses here contemplated. While it is perhaps unfortunate that his analyses do not deal with the bark and wood separately (as the proportion of bark to wood varies considerably between the tip and the base of a shoot) still his results, with that exception, are accepted as a working basis in this paper.

*Forcing Solutions through Shoots.*—In order to test the conduction capacity of ringed and bent shoots as compared with normal shoots, a very simple device was used (pl. 2, fig. 2). The height of the water column was four and one-half feet, which supplied a pressure of approximately one-seventh of an atmosphere. The stems were cut and adjusted to the rubber tubing under water to prevent air from getting into the open tracheae. The unit of measurement was the time required for two cubic centimeters of water to pass through the section of the shoot and to accumulate in the graduated stand-tubes.

*Microchemical Determinations.*—Sections were cut twenty microns thick on a sliding microtome. These were tested for starch with a potassium iodide-iodine solution. For sugars, the Flückiger reaction gave good results. It is a sensitive reaction and gives a test which is unmistakable. Care must be taken, however, to wash the

sections in distilled water, both before and after making the test, in order to rid them of sugars which, coming from the broken cells, might lodge in the other tissues and thereby give a false impression as to which tissues actually contain sugars.

*Microtechnique.*—The woody sections of pear and *Robinia* for permanent mounts were prepared according to the schedule given by Chamberlain,<sup>4</sup> using the paraffin method. It was necessary first to desilicify the wood tissue by immersing it in hydrofluoric acid (commercial strength) for several weeks.

In staining, safranin in combination with Delafield's haematoxylin gave very satisfactory results. Safranin or fuchsin with light-green, furnished good differentiation of xylem and phloem tissue.

*Ringing with the Aid of Chemicals.*—An effort was made to ring shoots without injuring the underlying xylem tissue. To do this it was necessary to prevent both mechanical injury and also drying effects.

Potassium hydroxide and zinc iodide, in varying percentages, were painted on the shoots in the form of a ring. In some instances the epidermis was gently scraped away before applying the solutions. In other cases the entire cortical parenchyma was cut away down to the pericyclic fibers, leaving only a thin layer of tissues surrounding the woody cylinder.

By microscopical examination of cross and longitudinal sections through the ringed areas, it was a fairly simple matter to determine if the phloem had been killed and the xylem uninjured.

*Nutrient Solutions.*—Dormant cuttings, approximately fifteen inches long, were taken from the middle of pear shoots in order to secure a length of stem which was fairly uniform in nutritive condition. Sets of twenty such cuttings were placed in solutions of sodium nitrate (0.5 per cent), sodium sulphate (0.5 per cent), glucose (0.2 molar) and distilled water. The cuttings were left in the solutions for two weeks and then transferred to distilled water. Where glucose was used, the solutions were renewed every other day.

In another experiment, solutions of sodium nitrate, glucose, and a combination of nitrate and glucose were forced into cuttings which were then immediately placed in distilled water.



## PRESENTATION OF DATA

The results of the investigation are presented under the following heads: Growth Responses, Starch Deposition and Depletion, The Presence of Sugars, The Effect of Ringing and Bending on Water Conduction, The Sieve-Tubes of the Pear Shoot, Permeability of the Tracheal Cross Walls, Injury to the Xylem Due to Ringing, Observations on *Robinia pseudacacia*, Ringing with the Aid of Potassium Hydroxide, and The Effect of Nutrient Solutions on Bud Growth.

## GROWTH RESPONSES

*Bending.*—About three weeks after the time of bending (July 9), many of the shoots produced secondary lateral shoots from the buds just below the bend. At the time of bending the shoots were still in active growth. Consequently, there was a geotropic response of the perceptive zone and growth again continued upward. This caused a second bend which seems to have had the same effect as a forced bend in producing lateral branches (pl. 1, fig. 1).

Perhaps laterals develop at or behind the bend because of some interference with the passage of food. Such an interference would necessarily be in the phloem because as a later experiment showed, bending does not interfere with the passage of materials through the tracheae.

*Defoliation.*—Defoliation of the upper halves of shoots on July 7 and 10 resulted within three weeks in the development of lateral branches from two or three distal buds. This was true almost without exception. Defoliation at later dates, July 28 and August 6, produced no such results. The growing period may have passed or perhaps defoliation at that date did not as radically disturb the nutritive condition as defoliation in the early part of July.

Harvey<sup>12</sup> found that the ratio of carbohydrates to nitrogen was reduced by defoliation, not only through the reduction of carbohydrates, but also through actual increase in the nitrogen content. This may be responsible for the pushing of the buds following defoliation.

The growth of buds the following spring on the defoliated parts was apparently like that on untreated shoots. The effect on bud



growth of defoliating half of a shoot did not carry over to the following growing season.

*Ringling*.—All ringling done before the last of July resulted in growth of the buds immediately below the ring (pl. 1, fig. 2). This was true regardless of whether or not defoliation accompanied the ringling, although the effect was most marked when accompanied by defoliation. Ringling after August 1 had no effect on bud growth until the following spring, at which time the buds below the ring developed into lateral branches.

*Heading-Back*.—The effects of heading-back are well known. The shoots cut back on July 20 produced growth from a few buds at the end, while those cut back on July 31, just eleven days later, remained almost dormant (pl. 2, fig. 1).

The date marking the end of the season within which buds of the Bartlett pear can develop seems to be well defined. That date was approximately August 1 at Davis, California, in 1923. The shoots cut back on July 20 and 31 show two very definite responses. There were no exceptions in either class.

Perhaps buds remain dormant after August 1 because of nutritive conditions. Hartwell<sup>11</sup> working with *Solanum* (potato), found that a retardation of growth in the plants was always accompanied by an excessive accumulation of starch in the tissues. The decrease of demand for starch, due to retardation of growth, might lead to this accumulation. However, he suggests that the retardation and cessation of growth may be due to carbohydrate congestion rather than that the accumulation is the result of growth retardation.

#### STARCH DEPOSITION AND DEPLETION

During the growth of pear shoots virtually no starch is present in the tissues except in the endodermis, which lies just outside of the pericyclic fibers and which always shows the presence of starch.

Almost immediately upon the cessation of length growth, starch deposition begins. It starts in the upper end of the shoot and continues progressively downward. It appears first, in appreciable quantity, in the medullary rays. The ray cells nearest the bark are filled first and then those inward toward the pith. Starch forms next in the pith and wood parenchyma cells and lastly in the bark, the cortical parenchyma cells being the chief storage tissue of this region.

The fact that the ray cells nearest the bark are the first to contain starch may favor the theory that conduction of carbohydrates is through the sieve-tubes, because these ray cells are the first ones available for storage of materials coming from that direction.

In the spring as growth begins, starch disappears first from the tip of the shoot and then progressively downward. This is the same order in which it is deposited during the summer. In the various tissues, however, it is removed in almost the reverse order from that in which it was deposited. The bark is emptied of its starch, first, next the medullary rays, the wood parenchyma cells, and lastly the pith. Neither the deposition nor the removal of starch follows this order sharply, for the action starts in one tissue before it is entirely completed in another. This indicates in general the order of deposition and depletion.

Sinnott<sup>23</sup> states that starch reduction in the wood takes place first and most extensively immediately around the tracheae. He suggests that this may not indicate carbohydrate conduction through these vessels but rather that the enzymes which convert the starch into a conductive form may themselves be carried in the water stream and hence come first into contact with the starch in the tissues surrounding the tracheae.

#### THE PRESENCE OF SUGARS

To determine the exact tissues which contain sugars is a difficult matter. If carbohydrates enter and withdraw from cells in the form of sugar, it is obvious that a sugar test should disclose its presence in practically all tissues. It cannot be concluded, therefore, that because a tissue contains sugar, it functions in the conduction of carbohydrates. However, both Mangham<sup>16</sup> and Czapek<sup>8</sup> report the finding of sugars in the sieve-tubes.

Numerous investigators believe that they have found sugars in the tracheae. From the tests for sugars, using Flückiger's reaction, the writer cannot conclude that the tracheae of Bartlett pear shoots contain these substances. In the preparation of a section for examination many cells are ruptured and their sugar content scattered over the whole section. It lodges in the irregularities of the tracheal walls and is not easily removed even by washing in distilled water. This gives a false impression as to what tissues actually contain sugars.

Nevertheless, in a general way, sugars can be located. They are most abundant in the cortical parenchyma cells. The wood parenchyma, the medullary rays, and even the pith contain appreciable amounts. The greatest amount, however, is in the bark. Proebsting<sup>21</sup> found that the bark of apple shoots contains from two to five times as much total and reducing sugars as the wood does.

THE EFFECT OF RINGING AND BENDING ON WATER CONDUCTION

Curtis<sup>6</sup> suggests that the inhibition of shoot growth at nodes below the terminal may be due to the inability of the lower buds to compete successfully for water.

To determine whether ringing or bending interferes with the passage of water and perhaps thereby causes the buds to grow just below the ring or the bend, the stems were subjected to pressure in the way already described, using the apparatus shown in plate 2, figure 2.

The normal relation between three consecutive sections of an untreated shoot was first established (table 1). The capacity is expressed in the number of minutes and seconds required for two cubic centimeters of water to pass through the sections. The sections requiring the shortest time for the passage of two cubic centimeters of water show the largest conduction capacity. The time was taken with a stopwatch. Where three consecutive sections were compared they were all of equal length. The length of sections was about 12 centimeters, except in the case of bent shoots, where a longer section had to be employed to include the bend.

TABLE 1  
THE CONDUCTION CAPACITY OF NORMAL STEMS

Stem No.	Upper Section		Middle Section		Lower Section	
	Minutes	Seconds	Minutes	Seconds	Minutes	Seconds
1	11	20	9	50	8	20
2	6	50	5	55	5	42
3	17	25	15	00	12	40
4	8	33	7	50	7	5
5	12	24	11	10	9	55

It is readily seen that the conduction capacity increases fairly regularly from tip to base, a circumstance which is due in all probability, to the greater diameter of the lower portion. This gives a basis for comparison in the tables that follow.

TABLE 2  
THE CONDUCTION CAPACITY OF RINGED STEMS

Stem No.	Upper Section		Middle Section (Ringed)		Lower Section	
	<i>Minutes</i>	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>
1	5	35	13	00	12	15
2	6	21	12	26	7	58
3	3	24	6	29	4	5
4	5	55	12	6	8	36
5	6	14	10	25	6	18
6	8	6	8	50	4	30
7	10	45	14	40	6	10
8	11	55	12	17	6	47
9	9	34	12	5	7	27
10	8	15	9	45	7	50

Ringling evidently decreases the conduction capacity of stems, in some cases as much as 45 per cent. In the first five examples the upper section shows greater conduction than the lower. This is due to the fact that the stem grew considerably more in diameter above the ring than below it. In the last five shoots the ring had completely grown over and the new xylem which had formed had in part overcome the inability of the ringed area to conduct water (pl. 5, fig. 1).

These results seem to favor the theory that water may be the cause of the buds developing below the ring. The following table shows, however, that in the case of bent shoots, something other than water causes the buds to develop.

TABLE 3  
THE CONDUCTION CAPACITY OF BENT SHOOTS

Stem No.	Upper Section		Middle (Bent) Section		Lower Section	
	<i>Minutes</i>	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>
1	6	37	4	50	4	20
2	8	00	7	12	3	50
3	6	12	4	39	3	50
4	6	55	4	37	3	28
5	10	40	6	33	4	39
6	5	24	3	45	2	56
7	4	38	2	8	2	29
8	25	43	20	50	15	10

(In this table the section containing the bend corresponds to the one containing the ring in table 2.)

The bent section shows no tendency to obstruct the passage of water. This does not favor the theory that water is the factor which causes the buds in the region of the bend to grow; nor that any substance which passes through the xylem is the cause. If we assume that buds grow as a result of a certain internal condition, it would appear that water is not the essential factor. The results indicate that we should look to the phloem rather than to the xylem tissue for an explanation of bud growth.

#### THE SIEVE-TUBES OF THE PEAR SHOOT

The sieve-tubes of one-year-old pear shoots lie in a rather well defined zone just within the ring of pericyclic fibers (pl. 6). They are, by virtue of their shape and structure, well adapted for conduction. Cross walls are few because of the great length of the tubes. It is difficult to determine their exact length on account of the frequent intersections of the tubes with medullary rays which extend out into the phloem. Their connections with the medullary rays suggest that important materials are conveyed by the tubes.

Palladin<sup>19</sup> states that a peculiarity of the movement of organic substances is that it is regulated exclusively by the activity of living cells and is the result of this activity. In other words he believes that this movement is controlled by internal conditions, while the movement of water from the soil, which admittedly goes on in the tracheae, is largely controlled by external conditions, such as light, humidity and wind. This fact along with certain anatomical features, he considers good reason to believe that the sieve-tubes, which are live tissues, rather than the dead tracheal tubes, are used for the conduction of foods.

The sieve-tubes of the pear shoot are without sieve-plates in the end walls. The cross walls are thin and plain without pits or pores. But in the side walls of the sieve-tubes there are numerous highly developed plates with large pores. Some writers term these plates "lattices." The fact that the end walls do not contain sieve-plates by no means indicates that the tubes are not adapted to longitudinal conduction. The sieve-tubes lie in a rather definite zone and overlap each other, thus enabling conduction to take place from one tube to another through the lattices much more rapidly than if there were sieve-plates only in the end walls, because there is more complete



contact by this arrangement than by an end contact of very narrow cells like sieve-tubes (pl. 4).

An attempt was made to determine whether bending injures or compresses the sieve-tubes, and thus causes an accumulation of food materials behind the bend, which results in the growth of those buds into laterals. Bark from the inside and outside of the bend was sectioned longitudinally, using the paraffin method, and compared with bark from above and below the bend. No significant difference could be observed between bent and unbent sections either in the condition of the sieve-tubes themselves or in their number. This does not necessarily mean that the tubes were not compressed or otherwise distorted when the bark was still on the bent shoots. The removal of the bark for sectioning probably released any tension present and the tissues might easily have resumed their normal condition.

#### PERMEABILITY OF THE TRACHEAL CROSS WALLS

The permeability of the tracheae to sugars and nitrogenous compounds was tested to determine the possibility of this tissue being a normal channel for these substances. The same method was used as in forcing water through stems to test their conduction capacity. The same shoots used in conduction capacity were tested in this experiment, thereby furnishing a comparison with the results obtained in using water. Glucose, a 2 per cent solution, and asparagin, a 0.4 per cent solution, were used as representing the most common transitive forms of carbohydrates and organic nitrogen, respectively.

The xylem allowed both glucose and asparagin to pass through. The solution of asparagin passed through the stems at the same rate as distilled water, but glucose moved more slowly, probably on account of its viscosity. The same comparative rate between ringed and unringed stems was found as when tested for water conduction.

In all cases the solution was tested for asparagin or sugar after having passed through the stem. This test was a qualitative one to detect if these substances were passing through the xylem tissue or were being withheld by cell walls or membranes. Distilled water gave a negative test for these substances after passing through the stem. This may indicate that normally there is little or no sugar or asparagin within the tracheae, for such material would be washed out by the water forced through the stem and would be detected by the qualitative tests.

Flood<sup>10</sup> showed that the liquid which issues from the hydathodes of *Colocasia antiquorum* does not contain sugars or other organic solutes and also that there is no filtering mechanism within the hydathode which might relieve it of such solutes. This, however, does not necessarily indicate that organic material was not carried in that xylem sap. Priestly and Armstead<sup>20</sup> forced sugar solutions of known concentration through stems and showed that tissues could remove sugar from the solution as it passed through. This action, they state, is reversible; that is, the solution may remove sugars from the stem. Whether it adds or removes sugars is dependent upon the relative concentrations of the tracheal sap and of the solution.

The tracheae of the pear are very small. They average approximately 0.1 mm. in length and not more than 0.02 mm. in width. The length of stem used in these forcing experiments was at least 10 cm. and often much longer. If each trachea was 0.1 mm. long, in a stem 10 cm. in length there would be 1000 cross walls to be passed by materials being forced through a single tracheal tube.

To determine if wood older than the current season's growth would permit glucose and asparagin to pass, these same experiments were repeated with *Eucalyptus* and *Prunus* (apricot and plum). The young xylem of three-year-old branches was cut away, leaving the two and three-year-old portion. This older xylem also allowed these substances to pass when subjected to pressure. Permeability of the tissues does not seem to be a factor which might allow the young xylem to be active in the conduction of organic materials while the older tissue is perhaps used only for water, as Dixon<sup>9</sup> suggests.

Branches of *Pinus* (pine), which has no tracheae but does have a corresponding conductive tissue in the tracheids, were tested by the same methods and were found to allow both glucose and asparagin to pass.

While these experiments do not indicate anything concerning the normal channels of these substances within the plant, they do show that as far as permeability of the cross walls is concerned, there is no objection to the theory that organic materials are conducted through xylem tissue as old as three years—unless it be true that the permeability is affected by cutting the shoots from the tree.

## INJURY TO THE XYLEM DUE TO RINGING

Ringling, plus defoliation above the ring, prevented any starch deposition in the part defoliated; whereas defoliation alone of the upper part of the shoot did not prevent starch deposition. This shows that ringling interrupts the upward movement of carbohydrates. From such experiments as these, Curtis<sup>6</sup> concluded that carbohydrates move exclusively in the phloem. He found that ringling interrupts not only the carbohydrate movement but also, to some extent, the movement of nitrogen.<sup>7</sup>

The objection to ringling, as a method of determining the tissue involved in food conduction, is that the outer xylem is unavoidably injured in the process. It may, therefore, be fallacious to conclude from the fact that ringling interrupts carbohydrate conduction, that carbohydrates move only in the phloem.

Since the outer xylem is injured by the process of ringling, it is important to know the extent of the injury. Longitudinal microtome sections through the ringed area show actual severing of the tissues by the knife used in ringling in addition to drying effects from exposure to the air. The tracheae appear to be clogged with materials and callus plugs for some distance into the wood past the extent of the knife injury (pl. 5, fig. 1). Measurements with an eyepiece micrometer showed that from 20 to 25 per cent of the xylem cross-section of one-year-old shoots was rendered non-functional for conduction as the result of ringling.

The inner xylem, however, is uninjured by ringling and is available for conduction. The continued growth of a shoot after ringling indicates that water must move through the inner xylem. The vessels of the pear do not develop tyloses as a result of ringling. To make certain that the technique used for determining the presence of tyloses was effective, the method was applied to *Robinia*, the classic example of a plant which naturally produces these structures within the tracheae.

If the xylem tissue is normally used for carbohydrate conduction, why should not all of the xylem in a current season's shoot be available for such a function? In that event, injury to the outer xylem alone due to ringling should not completely prevent this movement, as it apparently does.

Some of the rings grew over, the tissue regenerating from above and below. When this had taken place the growth of buds below the ring ceased, leaving short laterals of varying lengths. Moreover, the carbohydrates were again able to pass, which resulted in a good deposition of starch above the ring. It could not be determined whether the laterals stopped growth because the carbohydrates (and perhaps nitrogenous materials) again flowed by to the upper part of the stem or whether the natural end of the growing period, whatever its cause, had been reached. The fact remains that the growing over of the ring, the cessation of bud growth below this area, and the resumption of upward carbohydrate movement all occurred simultaneously.

Because carbohydrates again moved upwards after the ringed area had regenerated new tissue, it was first thought that this new movement must be through the phloem. However, longitudinal sections through the ringed area showed that both new phloem and new xylem had bridged the gap and that both sieve-tubes and tracheal tubes anastomosed through this region (pl. 5, fig. 1). This would indicate that carbohydrate conduction is restricted to the outer xylem or to the phloem or to both.

#### OBSERVATIONS ON *ROBINIA PSEUDACACIA*

This species was included in the discussion because it was found to exhibit features which contribute to the evidence on food conduction. Five and six-year-old branches were sectioned and tyloses were found to have completely clogged, without exception, every tracheal tube over a year old. Only in the outermost xylem the current season's growth—could tracheae be found free from obstruction. Not all of the vessels even of this new wood were open, but there were enough unobstructed tracheae to form a very definite area for conduction (pl. 3).

The efficacy of these tyloses as plugs was tested by attempting to force water through the stems. When the whole cross-section of stem was intact, water and also glucose and asparagin were easily passed through the tracheae. However, when the outer ring of unobstructed tracheae was cut away, leaving only the tylosis-plugged vessels, not even distilled water could be forced through.



Since tyloses so effectively block the tracheal tubes, this is further evidence that tyloses are not formed in the ringed or bent pear shoots, for water could be easily forced through those stems, even with the outer xylem cut away.

The formation and depletion of starch reserves in *Robinia* furnish interesting observational evidence as to the tissue involved. According to Atkin's<sup>1</sup> theory, carbohydrates are conducted into the xylem from the bark by the medullary rays and are stored in the wood parenchyma cells which form a sheath of starch-containing elements around the vessels. In the spring the carbohydrates in these parenchyma cells diffuse into the tracheae which they border and upward translocation through the tracheae takes place.

In *Robinia* the sheath of starch-containing elements around the vessels is marked, but as has been said, the vessels themselves are entirely blocked by tyloses. The carbohydrates, therefore, could neither enter the wood parenchyma cells nor leave them by means of the adjacent tracheae. Yet this parenchyma tissue is filled and emptied of its starch each year. In this species the path of the carbohydrates stored in the wood parenchyma must pass through the medullary rays to the phloem or to the very outermost xylem.

#### RINGING WITH THE AID OF POTASSIUM HYDROXIDE

The applications of zinc iodide to the shoots proved unsatisfactory as a method of killing a band of phloem. Wherever it was used it was carried upward in the bark and killed the shoot above the point of application. Strangely enough, the portion of the shoot below the point of application was always uninjured. It appears that movement took place only in an upward direction. The solutions were applied in the spring of the year.

Potassium hydroxide (7 per cent solution), when applied to areas where the cortical parenchyma had been cut away, killed the phloem in most instances, without perceptible injury to the xylem. It did not travel in the bark, as did zinc iodide, but remained in the tissue where it was applied.

This method of cutting away the cortical parenchyma before applying the solution of potassium hydroxide was the only one which gave the desired results. Merely scraping away the epidermis did not permit the potassium hydroxide to penetrate sufficiently far to kill



the phloem elements. Cutting away the outer bark without applying potassium hydroxide was unsuccessful because new phloem was developed from the cambium to replace that which drying might have caused to become non-functional. Where potassium hydroxide was used, this difficulty was overcome by frequent applications of the solution in order to kill any newly differentiating phloem elements. That the phloem was killed by this method was determined by microscopical examination. The sieve-tubes and their associated cells had collapsed and become discolored and seemed to adhere to the xylem in the form of a dry, protective covering (pl. 7).

Shoots were ringed in two places by this method and the buds removed between the rings in order that they might not use food in growth. As the shoots started to grow, the starch in the tissues between the two rings was not withdrawn to satisfy the demands of the growing regions above and below. Yet the xylem tissue which was whole and apparently uninjured was available in its entirety for the conduction of this stored food past the rings. The tissues above and below the ringed area were entirely depleted of their starch reserves shortly after growth had well started.

As a check on this experiment, portions of shoots were disbudded without being ringed. The starch in the disbudded regions was depleted by the growth of the buds above and below. It appears from this experiment that the non-removal of starch from between two rings is probably due to the fact that the xylem does not conduct carbohydrates.

It is of importance to note that this method of ringing with the aid of potassium hydroxide, in which the xylem is not injured, results in the growth of laterals from buds below the lower ring (pl. 5, fig. 2). Ringing, by removal of a band of bark down to the xylem, brings about the same response. Apparently ringing with the aid of potassium hydroxide is a method just as effective.

To say that starch between two rings never disappeared would be incorrect. Radial growth of the stem, which unavoidably takes place, slowly made demands on the stored foods in that locality. However, it required a long period for radial growth to deplete the starch reserves; whereas, in disbudded portions of shoots without ringing, the starch was rapidly withdrawn by bud growth above and below these regions.

Radial growth of shoots begins about the same time as bud growth. The new radial additions to the xylem and phloem can be observed within a few days after the buds start. As the apical buds start activity, radial growth in that region begins but it does not take place at lower regions on the shoot until the lower buds break. It appears that the conditions favorable to bud growth may also be favorable to radial growth.

#### THE EFFECT OF NUTRIENT SOLUTIONS ON BUD GROWTH

On February 12, four weeks after starting the experiments with nutrient solutions, the buds at the upper end of all the cuttings started to grow into laterals. At the same time the basal buds of the shoot treated with sodium nitrate started to grow, but basal shoots treated with glucose, sodium sulphate and distilled water remained dormant (pl. 8, fig. 1). The absorption of sodium nitrate by the basal portions of the cuttings probably lowered the value of the carbohydrate-nitrogen ratio by increasing the nitrogen factor. The growth of these basal buds cannot be attributed to proximity to the water for the basal buds of cuttings receiving other treatments did not grow at this time.

Three weeks later (March 2) the lower buds with the other treatments did make a weak growth. An examination of the starch content of the tips and bases of the cuttings showed that in every case the growth of the apical buds had been continued at the expense of carbohydrates in the base. The apical ends of the cuttings were still packed with starch in spite of the fact that the buds at that point had elongated several inches. The bases were generally entirely emptied of starch. This depletion of carbohydrates in the base by demands made by the tip might also lower the carbohydrate-nitrogen ratio.

In the second set of cuttings in which the solutions were forced through the stem and then placed in distilled water, approximately the same results were obtained with glucose and nitrogen as in the first experiment. In order of regenerative activity of the basal buds, the various treatments ranked as follows: sodium nitrate, sodium nitrate plus glucose, distilled water, and glucose. It is interesting to note that glucose alone forced into the stems at the basal end entirely inhibited the growth of the lower buds.

Where the nitrates and glucose were forced in together in equal parts, the ratio was probably lowered despite an increase in carbohydrates. A very little increase in nitrogen may lower the ratio materially. This is evidenced by the effects of relatively small applications of sodium nitrate on the growth and condition of large trees.

Sodium sulphate had no effect in bringing about the growth of basal buds. This would indicate that the response to sodium nitrate was due, not to the sodium, but to the nitrate radical.

Auchter<sup>2</sup> planted dormant privet cuttings so that half of the roots would grow in quartz sand while the other half grew in a rich loam soil. Growth started in the branches directly above the loam soil, while there was no growth on the quartz side until three weeks later. He concluded that the nitrogen in the soil was probably responsible for this early breaking of the rest period. His experiments show also that normally there is little or no crossing over of mineral nutrients or elaborated materials from one side of the plant to the other.

Harvey,<sup>12</sup> in studying the growth of apple shoots with special consideration of the rôle of carbohydrates and nitrogen, found that the tip of the shoot is higher in nitrogen, phloridzin, soluble solids, and water than is the middle or base of the same shoot. The base is higher in carbohydrates. He found that ringed shoots have less nitrogen, phloridzin, and soluble solids above the ring, but more carbohydrates. In other words, the chemical situation was reversed by ringing. Defoliation of a portion of the shoot, either basal or terminal, resulted in an increased nitrogen and a decreased carbohydrate content in that portion.

## DISCUSSION OF RESULTS

Ringing, bending, and notching, and other methods of injuring the bark, produce bud growth immediately below, rather than above the injury. This fact is significant, for it indicates that the response is probably due either to the absence of some substance or stimulus prevented from coming down past the injury or to the accumulation below the injury of some substance moving toward the tip. Because defoliation brings about the growth of buds into laterals, it seems that this growth may be associated with nutritive conditions. Defoliation, it should be expected, would decrease the amount of carbo-

hydrates. Harvey<sup>12</sup> found this was true, and also that the amount of nitrogen in the defoliated portion was increased by this practice. This brought about a lowering of the carbohydrate-nitrogen ratio which may have initiated growth. The carbohydrate-nitrogen theory of Kraus and Kraybill<sup>14</sup> is so susceptible of application to nearly all situations of this kind that it must be used with care. However, it seems to furnish a feasible explanation of the growth response to sodium nitrate.

Although various practices such as defoliation, bending, and ringing caused buds to grow, there was no response to these treatments after a certain period. No treatment after August 1 had any effect on bud growth. Perhaps, after this time, the elaborated materials start to move downward into the lower parts of the tree. More probably there are other factors operating which prevent a response so late in the season. This raises the question—does length growth stop and the terminal bud mature because the movement of organic foods is reversed, or do these foods reverse their direction because growth has ceased and the food is no longer needed to meet the demands of a growing tip? The latter hypothesis is more probable because starch is not deposited in the tip until after length growth has ceased.

Howard<sup>13</sup> is inclined to believe that rest sets in on account of the inhibition of enzyme activity which is due to an over-accumulation of the products of their work. Two factors, the approach of cold weather and the inhibition of enzyme activity by the accumulation of carbohydrates, act together, he states, to compel the shoots to cease length growth and mature the terminal bud. This theory shifts the responsibility for growth cessation to retarded enzyme activity rather than to carbohydrate accumulation and its relation to the nitrogen factor from a nutritive standpoint.

The fact that stimuli bring about growth responses in buds only within the period of the growing season, would suggest that perhaps the matter of dominance is related to the rest period which may, in turn, be dependent upon the nutritive condition as a result of carbohydrate-nitrogen relations. There is, however, a distinction to be drawn between the breaking of the rest period and the growth of the upper buds into laterals. The buds on the lower part of the shoot come out of the rest period and form a leaf or a cluster of leaves but normally do not elongate into laterals. There appear to be two factors,



or groups of factors, operating; one causing the buds to come out of the dormant condition, the other causing them to elongate into laterals. It is, nevertheless, not impossible that both of these responses are the results of nutritive conditions.

Since fruit buds are produced chiefly at the terminal ends of pear shoots (pl. 8, fig. 2), it is apparent that pruning, and especially heading-back, not only seriously decreases the bearing possibilities of the tree but also discards much of the growth made by the tree at the expense of food and energy. Perhaps pruning as it is commonly done, is an extravagant and wasteful practice. Bending, with a little judicious pruning, may prove to be a better orchard practice for pears. The food is thus diverted for the production of numerous fruiting laterals rather than for lengthy vegetative growth which must be cut back in order to keep the tree within a manageable height.

The appearance of fruit buds at the apical ends of the shoots seemed at first contradictory to the theory that buds near the apex form lateral branches as a result of a growth-favorable carbohydrate-nitrogen ratio. It appeared that there were two responses at the terminal portion of the shoot, vegetative vigor and fruitfulness, which are supposedly the results of two opposite nutritive conditions. A closer examination of the facts revealed that the two responses are in harmony with the carbohydrate-nitrogen theory. Harvey's<sup>12</sup> analytical results show that in the spring when the growth of buds is initiated, the tip portion of an untreated shoot is relatively high in total nitrogen and low in total sugars. Theoretically, this supplies the proper conditions for the growth of these buds into laterals. As the season progresses, the nitrogen in the tip decreases while the sugars increase until in the last part of June, just at the time when bud differentiation takes place, the sugar content of the tip is at its maximum. This furnishes a satisfactory explanation for the formation of fruit buds at the tip during late June, and also for the growth of laterals in this region at the beginning of the growing period.

The experiments in which solutions of glucose and asparagin were forced through ringed shoots indicate that so far as permeability of the tissues is concerned, there is no objection to the view that carbohydrates and organic nitrogen move through the xylem, unless, as has been pointed out, the permeability of the xylem tissue is altered when the shoots are cut from the tree.



Ringling, it was found, does interfere considerably with the passage of water. This interference is due to actual injury of the xylem tissue in the process of ringling. Were it not for this injury, ringling would be a simple and effective method of determining what tissues are involved in food conduction. One of the forms of injury to the xylem, suggested by Dixon,<sup>9</sup> is the formation of tyloses within the vessels. This work shows that no tyloses were formed in ringed pear shoots, although carbohydrate conduction was interrupted. The injury to the outer xylem elements occurring as a result of ringling was due to actual severing of the outer tissues by the knife, drying from exposure to the air, and clogging of the tracheae with substances deposited in them.

This injury, however, is confined to the outermost xylem, leaving the inner 75 or 80 per cent of the wood uninjured and available for conduction. The question arises, is there any reason to believe that the outer xylem of a current year's shoot may be used for organic materials while the inner portion is used only for water and perhaps minerals? In a branch several years old it may be that only the new xylem is active in conduction, but this distinction can hardly be drawn in the case of shoots of the current season where the xylem is all so newly formed.

The attempts to ring shoots without injuring the outer xylem indicate that conduction of carbohydrates probably take place only in the phloem. Shoots were successfully ringed with the aid of potassium hydroxide and the outer xylem was apparently unaffected, yet this tissue did not serve to move the starch reserve from between the rings.

## SUMMARY

On shoots of the Bartlett pear there is a marked tendency for the apical buds to grow out into lateral branches while those toward the base normally do not elongate.

Ringling, bending, and heading-back of shoots resulted in the growth of buds immediately below the ring, bend, or point of detachment. Any bud could be thrown into growth by these methods. However, no treatment of the shoot after August 1 would induce bud growth.

Defoliation of the terminal portions of shoots before August 1 always resulted in a prompt growth response of the buds in that portion. This fact suggests that nutritive factors are involved in the initiation of bud growth.

This belief was strengthened upon finding that a five-tenths per cent solution of sodium nitrate was effective in bringing about the growth of basal buds of excised shoots. Similar solutions of sodium sulphate had no such effect.

Ringings of shoots was found to interfere to some extent with the passage of water beyond the ring. However, bending, which also results in bud growth, did not interfere in the least with the rate of water passage through the tracheae. This suggests that neither a change in the water supply nor a change in the supply of any substance which might move in the tracheae is the factor which initiates bud growth.

If substances which move through the tracheae are not responsible for the initiation of bud growth, perhaps the responsibility can be laid to foods which move through the sieve-tubes. The investigation from this point, then, is contributory to a determination of the tissues concerned in the conduction of foods.

Starch deposition in shoots of the Bartlett pear begins very shortly after the cessation of length growth. Starch is deposited first at the tip of the shoot and then progressively downward toward the base. As growth begins in the spring, it disappears from the tip and then from the lower regions of the shoot successively. In the various tissues of the current season's growth, starch appears in the following order: medullary rays, pith, wood parenchyma, and bark. It disappears from the tissues as growth begins in the order: bark, medullary rays, wood parenchyma, and pith.

Micro-chemical tests for sugars show that the bark is much higher in sugar content than is the wood. The cortical parenchyma is the tissue abounding most in these carbohydrates.

Solutions of glucose and of asparagin were forced through excised shoots and were found to pass readily. This fact indicates that the tracheal cross-walls are permeable to these substances, and that so far as permeability of the tissues is concerned there is no objection to the belief that these foods move in the xylem.

Ringings of Bartlett pear shoots, by removal of a band of bark from the woody cylinder, interrupts the longitudinal movement of

carbohydrates. The objection to ringing as a method of determining the tissues involved in carbohydrate conduction is that tylosis formation and other factors may prevent the xylem from functioning in conduction.

Tyloses, when present in *Robinia pseudacacia*, effectively block the passage of water and solutions of glucose and asparagin through the tracheal tubes. Observations on tylosis formations indicate that the conduction of foods in this species is limited to the phloem or to the very outermost xylem. The inner xylem is completely obstructed by tyloses.

Ringing did not induce the formation of tyloses within the tracheae of pear shoots, but it did affect the xylem by actual mechanical injury and by clogging and drying of the outer vessels.

However, the inner xylem (75 to 80 per cent of the total) of ringed shoots was uninjured and was available for carbohydrate conduction but evidently was not used for this function, as ringing interrupted carbohydrate movement.

Both the new and the old tracheal tubes of normal excised pear branches allowed water and solutions of glucose and asparagin to pass through under pressure. This was determined by tests made after the removal of the radial growth of successive years. The results indicate that there is little reason to believe that the outer and inner xylem act differentially in the conduction of foods and water, i.e., that the outer xylem conducts only foods, while the whole cross-section is used for water transport. There is still less reason for believing this to be true of shoots of the current season where all of the xylem is so newly formed.

A method is described by which shoots were ringed without perceptible injury to the xylem, thereby overcoming the objection to the usual method of ringing. Even though the outermost xylem was uninjured, carbohydrate movement was interrupted just as though the shoots had been ringed in the usual way.

This study of the conductive tissues in shoots of the bartlett pear seems to indicate that, in this plant, the phloem is the tissue largely concerned in the longitudinal movement of foods and that there is a direct relationship of food movement to dominance of the apical buds, in that the nutritive condition from the carbohydrate-nitrogen standpoint is presumably involved.

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### PLATE 1

Fig. 1. A pear shoot, bent July 10; photographed August 15. The bend near the tip is due to geotropic response but appears to have the same effect on bud growth as the forced bend. Note the lateral growth behind each bend.

Fig. 2. A pear shoot ringed in March. Vigorous growth was produced from a few buds below the ring. Shoots ringed after August 1 did not show this characteristic response until the following spring.



Fig. 1

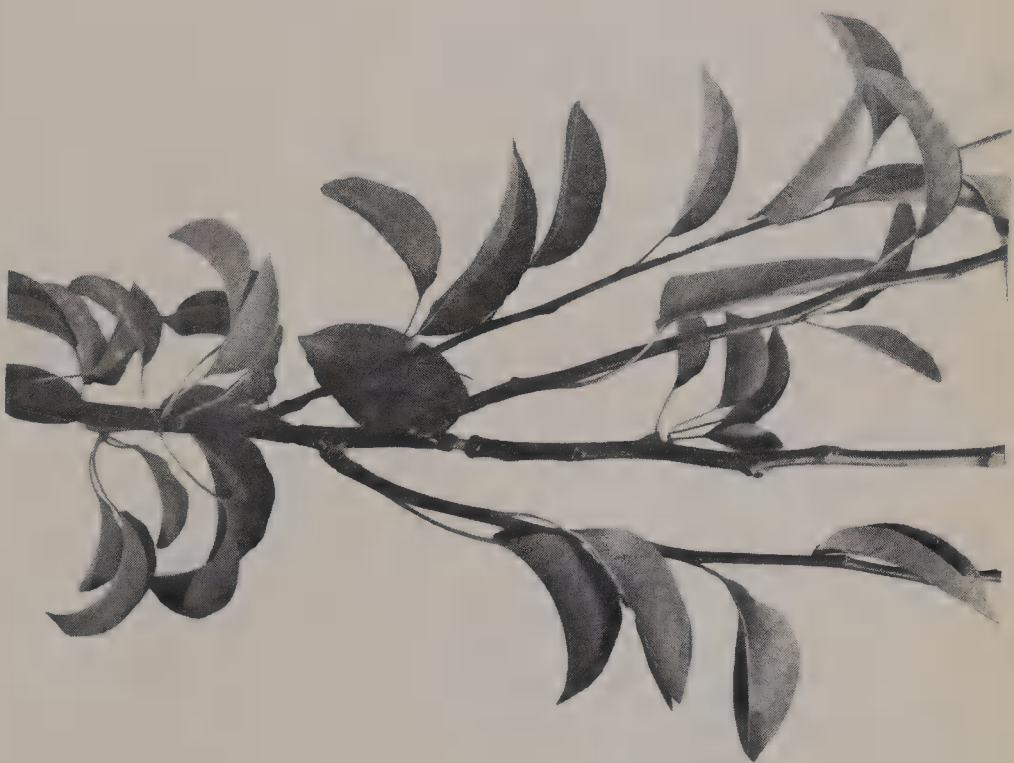


Fig. 2







## PLATE 2

Fig. 1. Pear shoots headed back on July 20 (left) and July 31 (right), just eleven days apart. This indicates the end of the period within which buds may elongate into lateral branches. Defoliation, bending, ringing, or heading back produced no growth response after August 1, at the University Farm, at Davis.

Fig. 2. The pressure device used to force solutions through the shoots to test their conduction capacity and permeability. The height of the liquid column was four and a half feet. The sections of stems were cut and adjusted to the rubber tubing under water in order to prevent air from entering the tracheae.

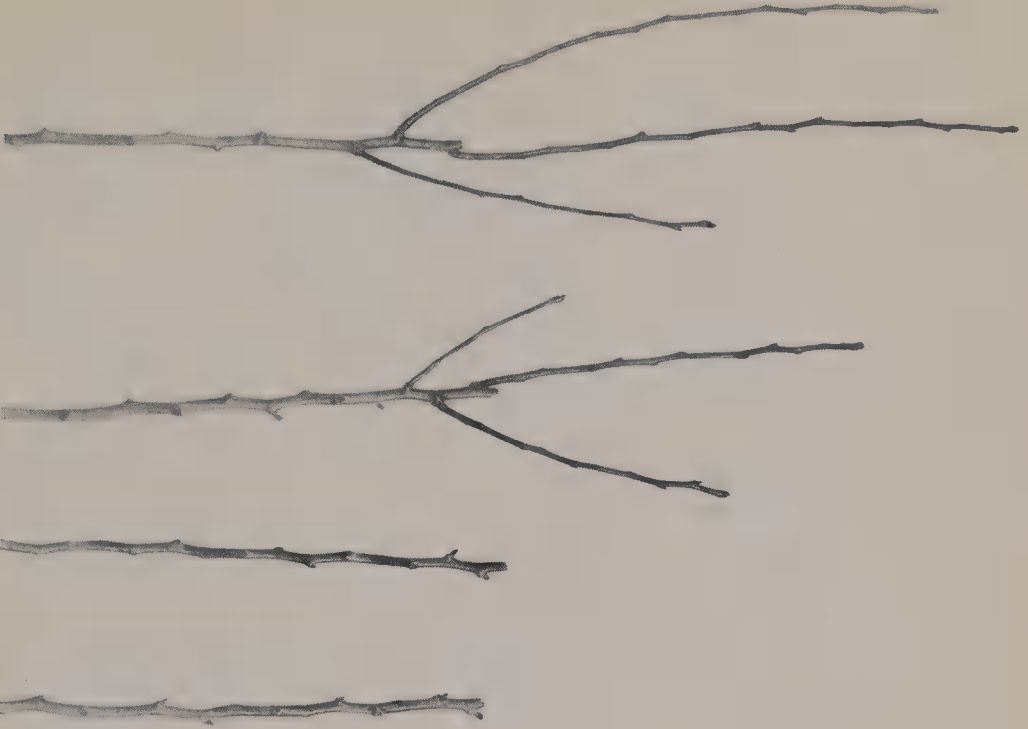


Fig. 1

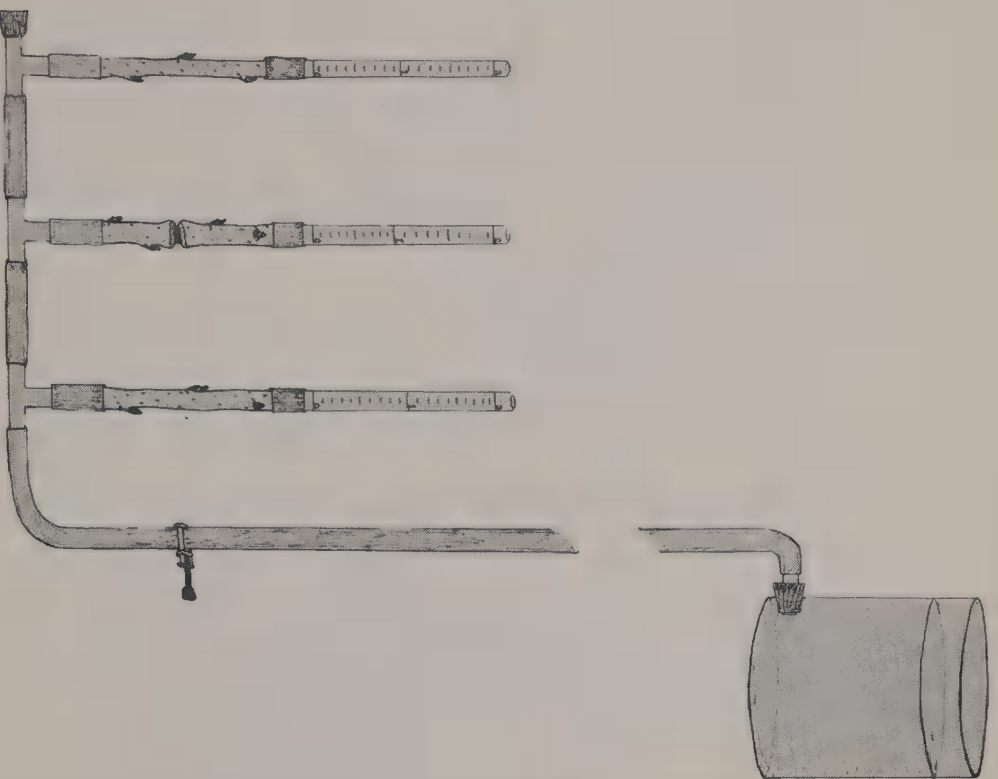


Fig. 2

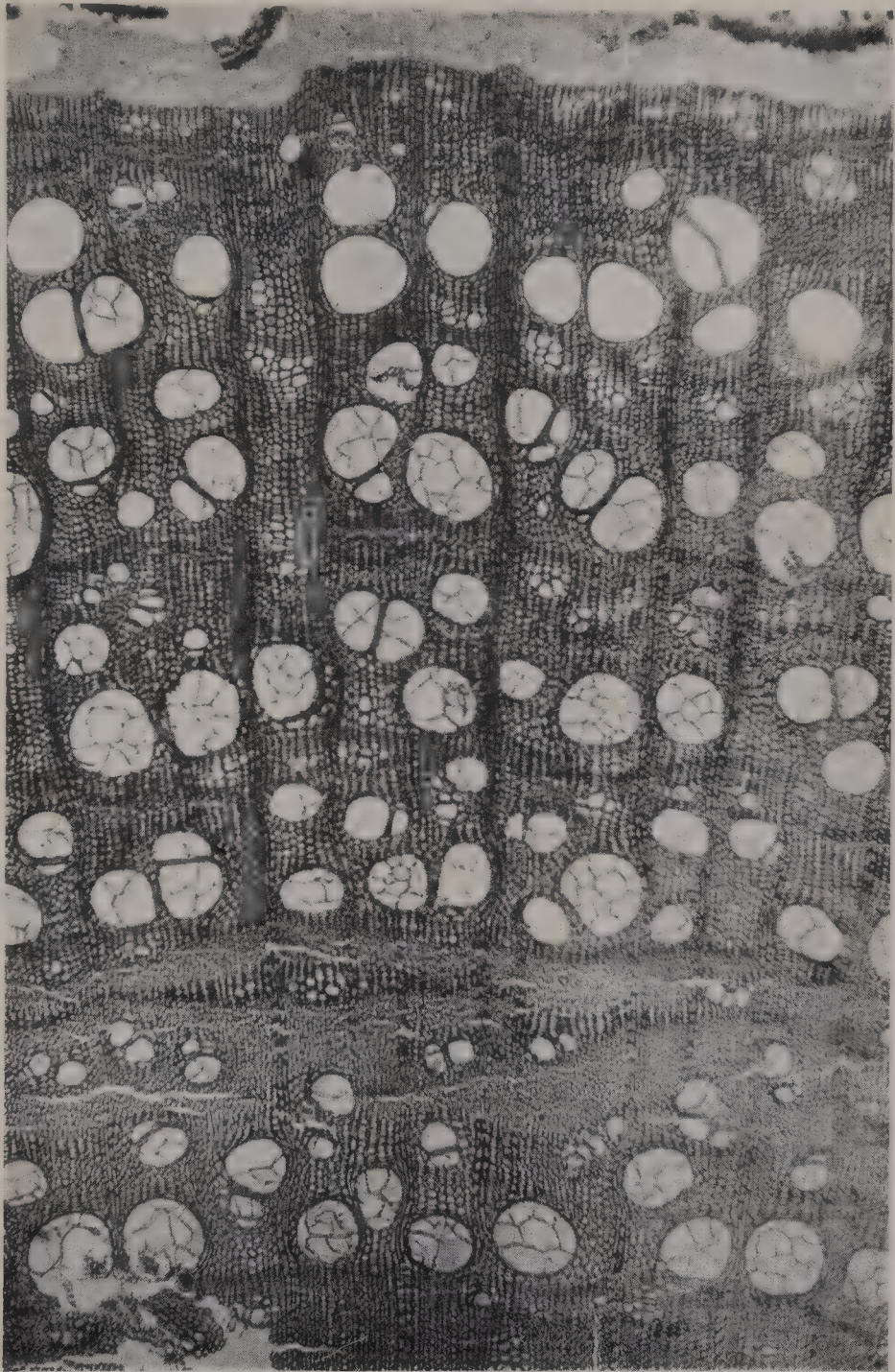




### PLATE 3

A cross-section of a six-year-old stem of *Robina pseudacacia*. Note that tyloses completely block the tracheal tubes except in the current season's growth. These tyloses form an effective barrier to materials which might otherwise move through the tracheae. In this species the conduction of foods is necessarily limited to the phloem of the very outermost xylem.









#### PLATE 4

A longitudinal section of a pear shoot showing the sieve-tubes. Note their adaptability for conduction—their length and the highly developed “lattices” in their side walls. The medullary ray cells are shown extending into the sieve-tube area.











## PLATE 5

Fig. 1. A longitudinal section of a pear shoot taken through the ringed area which has completely healed over. Both new phloem and new xylem have been regenerated. Note the injury to the xylem made by the knife in the process of ringing. Note also the clogging of the tracheae in this region while the inner xylem is uninjured and unobstructed. Yet this inner xylem is not used for carbohydrate conduction. The question arises, Is the outer xylem normally used for this function?

Fig. 2. Pear shoots which were ringed in two places by cutting away the cortical parenchyma (leaving the pericyclic fibres) and by applying from time to time a seven per cent solution of potassium hydroxide. The area between the rings was disbudded to prevent the removal of starch by bud growth. The buds below the lower ring are starting to grow.

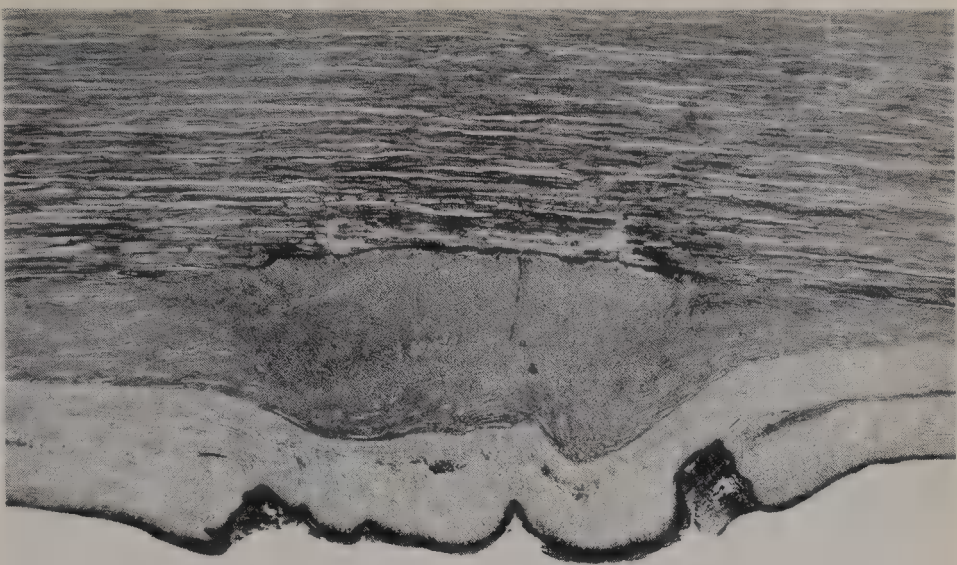


Fig. 1



Fig. 2







## PLATE 6

A cross-section of a normal pear shoot. The sieve-tubes lies in a rather well defined zone between the sambium and the ring of pericyclic fibers. Interruption of carbohydrate movement was accomplished by cutting away the cortical parenchyma (to the pericyclic fibers) and painting the wound with potassium hydroxide in order to kill the phloem elements.













## PLATE 8

Fig. 1. Pear cuttings which were placed in a one-half per cent solution of sodium nitrate. Note that the basal buds are starting to grow. In the solutions of glucose, sodium sulphate and distilled water, the basal buds remained dormant. Since sodium sulphate did not cause the basal buds to grow it would appear that the response to sodium nitrate was due, not to the sodium, but to the nitrate radical.

Fig. 2. Typical shoots of the Bartlett pear, showing the location of flower-buds. It is from this same locus on the shoot that lateral branches are produced. It is interesting to note that buds containing flowers start activity long before the purely leaf-buds on the lower portion of the shoot.



Fig. 1



Fig. 2

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